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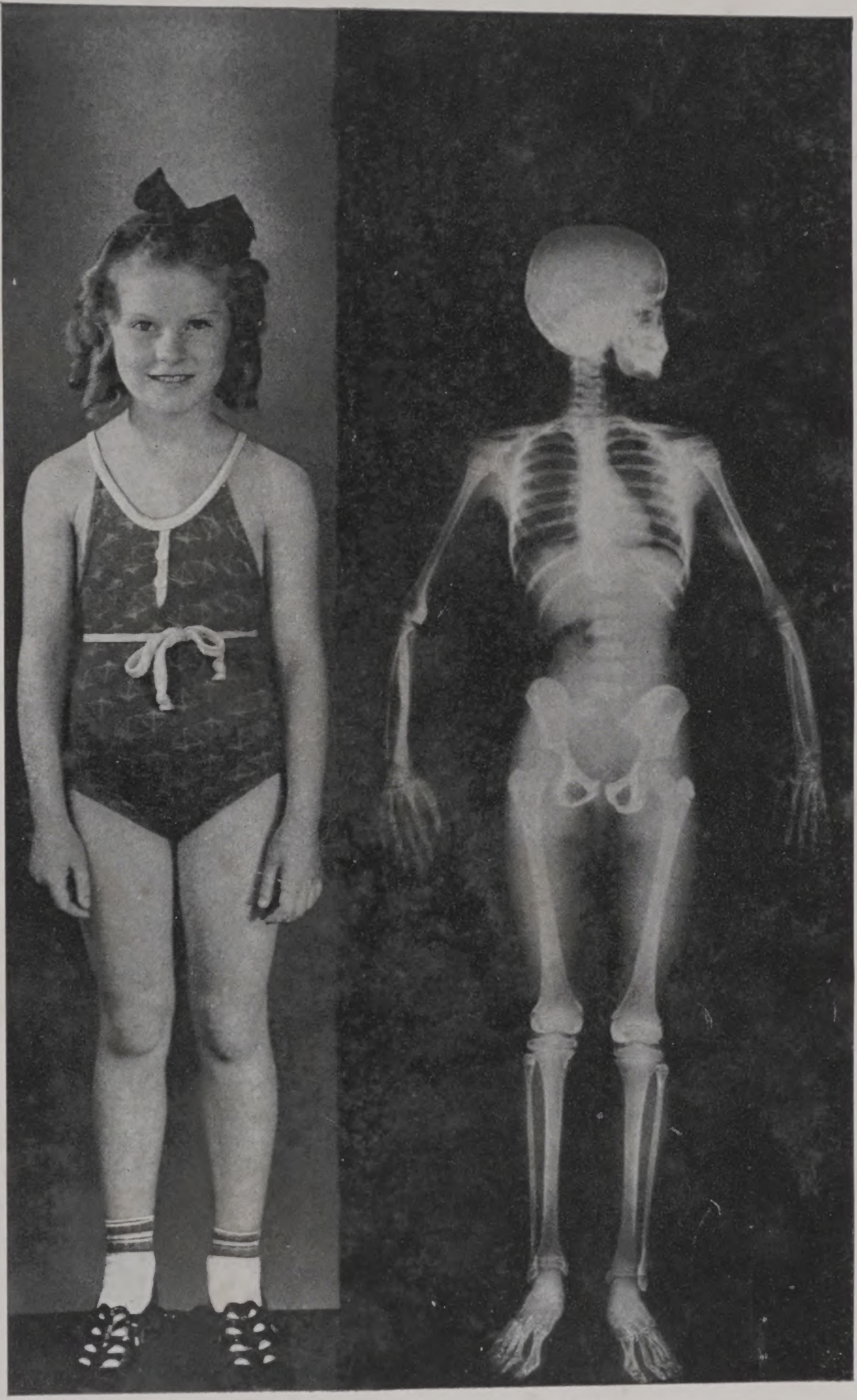
NUTRITION
AND
CHEMICAL GROWTH
IN CHILDHOOD

VOLUME I
EVALUATION



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NUTRITION
AND
CHEMICAL GROWTH
IN CHILDHOOD

VOLUME I
EVALUATION

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With a FOREWORD by
HUGO A. FREUND, M.D., PRESIDENT
BOARD OF TRUSTEES, CHILDREN'S FUND OF MICHIGAN

NINETEEN HUNDRED FORTY TWO

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Nutrition and ch

Dedicated to

THE MANY WHO HAVE LABORED INTENSIVELY
AT GREAT PERSONAL SACRIFICE
THAT THESE FACTS MIGHT BE ADDED
TO OUR SCIENTIFIC KNOWLEDGE

THE CHILDREN'S FUND OF MICHIGAN

Established April 11, 1929, by James Couzens

"TO PROMOTE THE HEALTH, WELFARE, HAPPINESS AND DEVELOPMENT OF THE CHILDREN OF THE STATE OF MICHIGAN, PRIMARILY, AND ELSEWHERE IN THE WORLD"

—From the Trust Instrument by James Couzens

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FOREWORD

TWO volumes on nutrition and chemical growth in childhood represent the accumulated experience and efforts of Dr. Icie G. Macy Hoobler and her associates. Here is the record of a group working as a unit under the guidance of and stimulated by the thinking of one who has devoted over twenty years steadfastly to scientific investigation of the processes governing fetal life and early childhood. Independent contributions of her co-workers have added important observations and are included. The singular devotion of all to exploration of the earliest processes of development in the human organism has made this publication possible at this time.

Dr. Macy Hoobler began her work about two decades ago in a small laboratory jointly provided by the Merrill-Palmer School and the Children's Hospital of Michigan. During that period, studies pertaining to maternal metabolism during the reproductive cycle and to the secretion and composition of human milk were stressed. As time went on, physicians, as well as biologic chemists, became familiar with much that was accomplished, through articles that flowed from the investigations and were printed in numerous publications.

The reports of this group have been published in so many clinical, as well as scientific journals and archives, and have been editorialized in so many publications that it would be quite impossible for the pediatrician, or the practising physician, to have access to any considerable portion of the literature. This work, however, is more than a compilation. It contains many of the very recent studies of Dr. Macy Hoobler, and investigations initiated by her associates. These original biological and chemical investigations cover particularly that portion of their work dealing with the development of children between the ages of 4 and 12. As a result we have a mosaic for those who would visualize the early chemical processes of life and the progressive changes expressed in terms of the actual metabolic activities of the normal child.

It need not be emphasized that a knowledge of biochemical and metabolic processes is essential before an understanding of deviations from the normal, and pathologic changes, can be properly evaluated by the practitioner of medicine. The publication of this work in monograph form has, therefore, been undertaken so that physicians and research workers, alike may have at their disposal, not alone a reference work of normal values, but also integrated information useful for practical application. In the first volume, *Evaluation*, the scientific material, up to the present time, represents the work accomplished in complete form. The second volume, *Interpretation*, will emphasize the practical value of the factual data. It is for this reason that those who have created the work have gone on to the task of presenting it in a second volume.

The Children's Fund of Michigan, created by the late Senator James Couzens, takes pardonable pride in the printing of these books. The publication of this work fits in with the objectives of the Children's Fund of Michigan, both from the standpoint of studying the development of the child, and also for the purpose of making additional knowledge available to those who have the responsibilities of guiding children to a healthy adult manhood and womanhood. The funds provided for the investigations and the publication of these volumes are small in comparison with the wealth of new information developed and the many opportunities afforded those whose interest lies in uncovering new knowledge about childhood. It is hoped this monograph will create a wide interest and be a continuing stimulus to those who have participated in its preparation.

Hugo A. Freund, M.D.

Detroit, Michigan

PREFACE

THE sciences concerned with man as a living organism have made enormous progress during the past twenty years through investigations on human beings, though much of the work has been by the trial and error method, with studies conducted in homes, hospitals and other social institutions. My collaborators and I have had the privilege and pleasure of pioneering in several important phases of biological research. Indeed, over many years we have devoted a full quota of time and effort to some of the most difficult and tedious problems presented by the processes involved in the life cycle of man. Many times we have groped in the dark to discover scientific truths. No task has been too great, no technique too laborious for us to willingly undertake for the sake of securing a fuller understanding.

As a result of these concerted and sincere efforts, it is hoped that from the present record of scientific investigation of the results of life's processes, other investigators with hopes and ambitions similar to ours may find assistance and guidance in the projection and pursuit of plans that will result in the accumulation of additional knowledge. Our knowledge is elementary and will advance only through the accumulation of many types of scientific data, on numerous human beings, in various physiologic states, conditions and stages of development.

The inspiration which impelled this Laboratory's pursuit of understanding human performance came from such great teachers as Lafayette B. Mendel, Julius Stieglitz, Graham Lusk, T. B. Osborne, and Mary Swartz Rose. The creation of the Laboratory was made possible by Edna N. White, Director of the Merrill-Palmer School, and Margaret A. Rogers, Superintendent, Children's Hospital of Michigan. The establishment of the program and pursuit of its objectives were accomplished through the efforts of Julia Outhouse, my first associate, and B. Raymond Hoobler, then Director of the Medical Staff of the Children's Hospital of Michigan. All of these have left the imprint of their keen minds upon this work of many years. The personal contacts, writings and kindly guidance of E. V. McCollum, Robert C. Lewis, John R. Murlin, Eugene F. DuBois, Joseph C. Brennemann, Sir Edward Mellanby, Lady May Mellanby, Alfred T.

Shohl, Henry C. Sherman, H. H. Mitchell, Lydia Roberts, Amy Daniels, P. C. Jeans, Genevieve Stearns, and Donald D. Van Slyke have assisted me in the arduous task of planning and carrying forward to completion the studies which are to be recorded in this and a forthcoming volume.

For more than twenty years we have delved for knowledge of the processes by which the human body enlarges itself and maintains life. During the early years we investigated the phenomena of the maternal reproductive cycle, the mineral metabolism of mothers during various stages of pregnancy and lactation, and the composition and secretion of human milk. During these years, also, we investigated the metabolism of normal women. For the past ten years we have dedicated a major portion of our resources to the study of normal children between the ages of four and twelve years, although other, extensive, related studies have been carried on during the same period, notably concerning the growth of infants and the chemistry of the red blood cell.

In our attempts to expand scientific knowledge of life processes we have not hesitated to enlist the knowledge and methods of other sciences and we have sought the assistance of those especially qualified in particular phases of the investigations. We have been firm in the belief that a single approach to a question involving the functioning of the human body cannot provide adequate information, that a narrow approach to such broad problems may be productive of misinterpretations. Although the processes of nutrition and growth involve chemical reactions which result in the incorporation of new tissue into the body, either as addition or replacement, chemistry alone cannot provide complete information. Thorough understanding must include evaluation of the many influences which may predetermine, alter, or control the efficiency of the processes themselves. For this reason we have emphasized the value of making numerous types of determinations and a variety of observations.

Investigation of nutrition and chemical growth in childhood has included study of the intake and excretion of 18 chemical substances by normal children, supplemented by observations and determinations which may be classified as pediatric, anatomic, psychometric, physiologic, anthropometric, roentgenologic and chemical. The data were accumulated under highly favorable conditions with the best available techniques, by competent investigators in well equipped laboratories, supplied with the latest and most efficient instruments for the highest type of scientific work. Even more important, the subjects were willing, cooperative, and trained in all procedures concerned in the collection of metabolic specimens and of physiologic data. Their habits and environment were regulated and arranged to stimulate the most healthful living, with emotional stress and strains reduced to the minimum.

The experience of many years has produced techniques which are efficient and economical in conducting investigations with human subjects; accurate chemical and physicochemical methods which conserve the time and energy of the laboratory analyst; simple, reliable methods of recording voluminous data; and, improved methods of handling and interpreting the values obtained by experimental procedures. The intrinsic worth of the various methods available for integrated assessment of the phenomena of growth and metabolism have been studied and those adopted which seemed to offer the greatest advantages to obtaining extensive, interrelated interpretations of the data upon our subjects in their particular environment, by our staff, with the laboratories, equipment and funds at their disposal. With the application of polarographic and spectrographic methods of determination to biological materials, we have approached an accomplishment I have long sought—rapid microanalysis in metabolic balance investigations. As this end is attained, the balance method of study is brought within the realm of medical practice. When the analytical values from metabolic balances can be procured a few days after the collection of samples, the method will be of great practical value in diagnosis and in following the course of disease before and after treatment.

Together with representative data from the research carried on by the Laboratory throughout the years, these techniques, methods and procedures provide the basis for Volume I, *Evaluation*, of *Nutrition and Chemical Growth in Childhood*. While no claim is made for completeness in scope, the methods presented include those of the usual types of biological studies, together with other, equally important, investigative procedures which have been included in our own inquiry.

To draw a satisfactory line of demarcation between the material presented in Volume I and that assigned to Volume II has been difficult. The techniques and methods are readily separated from the voluminous results and attendant interpretation, but their presentation would be incomplete without data which demonstrate the experimental control possible with the procedures and the accuracy attainable with the analytical methods. In giving general results in Volume I we have included, also, representative data illustrating the performance levels of normal children and the possible relationships which must be considered in the interpretation of experimental results obtained with living human subjects.

In the present volume, *Evaluation*, emphasis has been placed upon details of technique and the average and range of performance by normal children at different age levels. Children who were studied at more than one age level, several years apart, are considered as different individuals in each investigation. Volume II, *Interpretation*, will consider the nutrition and growth of individual children and will emphasize the synergic and cumulative aspects of nutriment assimilation and the chemical composition of growth.

When we have applied statistical treatment to the data, it has been in an honest effort to clarify the biological issue, not to strive for greater accuracy than the conditions of the experiment warrant. No pretense is made for completeness of mathematical exploration and evaluation of the data, for only those phases which seemed most helpful and necessary in the biological survey of the methods used and data obtained have been subjected, thus far, to bio-statistical advice and interpretation. Comprehensive statistical treatment of all the data procured by the Laboratory would require a separate volume and must be left as a future project.

In presenting the aggregation of results accumulated during the years we have had to enter into discussions embracing a majority of the chemical and medical sciences. The written records of hundreds of excellent research endeavors have been perused and the facts and thoughts obtained from the records have left their imprint upon this work. To review the literature in these many fields would be a herculean undertaking, a task precluded by considerations of space and time and rendered unnecessary by the reviews available which have been compiled by authorities in their respective sciences. One of the largest vacancies in review literature was recently filled by Shohl's[312] excellent *Mineral Metabolism*. For these reasons we have used only such references as are necessary to the presentation.

Very little comparison of our data with those in the literature has been attempted, largely owing to the incongruity of conditions and methods of study. The only study which approaches this one in scope was conducted by Guy Clark[48] and included balances for the positive and negative minerals, but the subjects were adults. The records from our own investigations have consistently pointed out the many, widely diverse influences which determine physiological responses and familiarity with the implications contained in the data has impressed the advisability of letting the results remain, for the most part, unclouded by attempts to reconcile inherently dissimilar observations.

The projection and completion of the work herein recorded have been accomplished only through the whole-hearted participation of many individuals and organizations. Over forty chemists and more than thirty practicing physicians have been involved in the program, as members of the staff or as enthusiastic collaborators. We are especially grateful to Hugo A. Freund, M.D., President; William J. Norton, Sc.D., Executive Vice-President and Secretary; and the other members of the Board of Trustees of the Children's Fund of Michigan, whose constant interest and encouragement have made this presentation possible.

The material upon nutrition and chemical growth could not have been accumulated and the projects carried to successful completion without the continuous, whole-hearted support of a cooperative group of chemists. Helen A. Hunscher, Ph.D., Frances Cope Hummel, M.S., and Betty Nims Erickson, M.S. shared in the planning of the studies. Dr. Hunscher was an invaluable assistant in the administration of the early study regimes. The objective efficiency of Frances Hummel and the late Betty Erickson was of inestimable value in both the design and accomplishment of the analytical procedures, and their well-rounded experience in scientific techniques contributed in great measure to the scope and accuracy of the investigation.

Since 1938, Harold H. Williams, Ph.D., assistant director of the Laboratory, has contributed immeasurably to the successful conclusion of the investigation. Various phases of chemical investigation have been supervised by Dr. Williams and by Eliot F. Beach, Ph.D., and Adolph Stern, Dr. Ing. Dr. Williams has been responsible for determinations in the field of lipid chemistry, Dr. Beach in the field of protein chemistry; both have contributed greatly to the integration of the manuscript. Dr. Stern has developed and applied the polarographic and spectrographic methods of mineral analysis.

A large portion of the analytical data has been obtained through the laboratory efforts of Margaret E. Coryell, Ph.D., Priscilla Bonner Horton, A.B., James Horton, B.S., Mary Bates Olson, M.S., Marion Lange Shepherd, A.B., Helen J. Souders, M.S., and D. Maxwell Teague, M.S. Ira Avrin, M.S., Louise Emerson Bound, B.S., Eva Donelson, Ph.D., Elizabeth Endicott, B.S., Harry Galbraith, B.S., Olive D. Hoffman, Ph.D., A. Theresa Johnston, A.B., Lan Chen Kung, Ph.D., Bertha Munks, B.S., Helen Sternberger Slyker, Ph.D., Camilla Stephens, B.S., and Alice Noll Walsh, B.S., assisted in the analytical procedures.

Harriet J. Kelly, Ph.D., statistician, has supervised the calculations, devised a most efficient method of recording the final values from the studies, and applied statistical treatment to the data, assisted by Marion Tobey and Margaret Garner.

Ralph E. Sloan has prepared the manuscript for publication, with the assistance of Francis J. Slyker, Ph.B., illustrator. The major burden of the secretarial work has been carried by Esta Fulton Beach, Frances Emmons, A.B., Rose Schwartz Sugar, A.B., and Alice Stieglitz.

Many clinical techniques were employed in the studies of nutrition and chemical growth. This phase of the investigation was made possible by the interest and assistance of Samuel S. Bernstein, M.D., Breton Hamil, M.D., Fanny Kenyon, M.D., Marsh W. Poole, M.D., and the late Charles A. Wilson, M.D., pediatricians; Pearl Lee, M.D., hematologist, Children's Hospital of Michigan; Alberta Maloney, D.D.S.; and Helen King, physiotherapist.

Many other individuals have given freely of their time and energy that the scope of the investigation might be as broad as possible and the results have greater accuracy and practical value. The splendid cooperation of Frances Knight, director, and the staff of the Methodist Children's Village, Detroit—where the children lived healthy, well-ordered lives—contributed immeasurably to the accuracy attained in the studies.

We are deeply appreciative of the courteous participation in this work by the individuals who have collaborated within the domain of their specialized sciences. Lawrence Reynolds, M.D., roentgenologist, Harper Hospital, Detroit, interpreted the roentgenograms of the children, made the fluoroscopic examinations, conducted the roentgenographic studies of gastrointestinal motility, and supervised the Laboratory's roentgen technician, Rachel Moore. The late T. Wingate Todd, M.D. and C. C. Francis, M.D., Department of Anatomy, Western Reserve University Medical School, made the assessments of *Differential Skeletal Maturation* according to the technique developed by Dr. Todd. Maud E. Watson, Ph.D., Director of the Child Guidance Division, Children's Fund of Michigan, consulted with the author, with the psychologists and with the psychiatric social worker. Nina Ridenour, M.S. and Jeanette McClure, M.S., psychologists, estimated the intelligence quotients of the children, and Verna J. Sloan, M.S., psychiatric social worker, observed the children with the view of establishing their emotional reactions to the studies.

Much of the tedium of this project has fallen upon those who have, in addition to already pressing programs of their own, generously given time and energy to reading the manuscript for this volume. I greatly appreciate the contributions to this presentation which have resulted from careful, constructive criticisms of the material, by Helen A. Hunscher, Ph.D., L. A. Maynard, Ph.D., Henry C. Sherman, Ph.D., and W. R. Bloor, Ph.D.

If we have contributed to the enlargement of scientific knowledge of nutrition and growth in childhood, our hopes and ambitions will have been fulfilled and our effort and expense justified. Perhaps others can profit by our varied experiences and perchance open the way to new channels of more exhaustive and fruitful research in the future. Meanwhile, the methods and values recorded here may serve as useful aids in the diagnosis and treatment of disease as well as a basis for proper care of normal human beings.

ICIE MACY HOOBLER

June, 1942
Detroit, Michigan

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NUTRITION
AND
CHEMICAL GROWTH
IN CHILDHOOD

VOLUME I
EVALUATION

CHAPTER I

INTRODUCTION

ANY inquiry into the vital chemical processes of maintenance or growth necessarily is accompanied by observations on nutrition, which comprises the resynthesis of proteins, fats, carbohydrates, inorganic salts, water and vitamins into newly organized body tissues as they are replaced and increased in mass; the science of nutrition is a study of food substances and the biological processes which utilize them.[206] A fund of information concerning growth, nutrition and metabolism has evolved during the past two generations, yet, because of the vastness of the subject, our knowledge remains fragmentary. Moreover, knowledge of the chemical composition of food and the selective action of the body in the digestion, absorption, and utilization of the ingested foods have not, as yet, been fully elucidated. Extreme variations in the daily diet must require marked compensatory actions on the part of the body.

The diverse mechanisms involved in the intricate, individual responses of human subjects of physiological and biological investigations have been recognized for many years. The difficulties inherent in any attempt to use the human organism for experimental work were summarized by Rietz and Mitchell[278] in 1910 almost as well as would be possible today:

> It is a generally recognized fact that two men to all appearances under identical conditions and of similar size, physique, constitution, temperament, etc., will often, on experimental investigation, yield considerably discordant results as to utilization of the food, composition of the feces and urine, nitrogen, phosphorus and sulphur retention, water elimination, changes in body weight and, perhaps more especially, structural composition of the blood, resistance to toxemia or bacteremia, intestinal bacterial flora, composition of the digestive juices, rate and character of digestion, etc. Again, it is well known that the same individual organism, under apparently identical conditions, will not behave in an identical manner at different times, variation being more or less pronounced depending upon the phase of metabolism considered. Such physiological behavior, peculiar to each individual organism and neither amenable to prediction or control, is ordinarily collectively designated as "individual idiosyncrasy." This property of living organisms responding differently within more or less wide limits

to identical conditions in so far as we can control conditions, we shall designate by the expression "functional variability."

The above paragraph was introductory to a discussion of metabolism experiments and their interpretation by statistical methods. The discussion presented 30 years ago is still fundamentally accurate and the subject, instead of being clarified during the last three decades, has been broadened by the discovery of many additional contingencies which today are recognized as necessary considerations in the interpretation of biological data.

It is difficult to make detailed observations on man because he does not countenance experimental control and conditions that regulate his existence and environment beyond a period of a few days. In order to obtain a meaningful description of the patterns of changing structure and function one of two courses may be followed. A few simple, pertinent tests may be made on sufficiently large numbers of individuals to justify a statistical evaluation of the results and thereby obtain the cross-sectional type of data but in view of the present lack of knowledge concerning the chemistry of tissue replacement and growth and the chemicophysiological criteria for identifying and evaluating its progress in the living organism, we have followed a more fruitful course of attack on the problem. By observing groups of normal individuals under consistent environmental conditions, at regular intervals, using simultaneously a battery of chemical, physical, clinical and nutritional tests, we have been able to employ improved methods for determining the developmental changes which take place in sequence within the individual as he increases in size and matures in structure and function. Such series of measurements yield the longitudinal type of data and permit broader interpretation, with its larger implications. Dearborn and Rothney[65] have estimated that "in the case of standing height, the available data on only 248 cases represent the equivalent of cross-sectional measurements on 270,000 cases."

We have no way of quantitatively or qualitatively measuring the rate or extent of an individual's past progress through biological time. The most profound effect upon the individual's *capacity* for growth and development is exerted by his parents. This effect is the sum of his genetic inheritance and the influences of the many factors involved in intrauterine life. We have no objective method of estimating the influence contributed by these sources at any time. Even a maximum of familiarity with the individual's and his progenitors' life histories provides no concrete measurement. In selecting human experimental subjects, we cannot separate the physical and mental characteristics of an individual and attribute them to their respective causations, but must accept as a normal individual one who conforms to accepted standards of growth, development, nutrition and behavior and possesses no detectable manifestation commonly considered abnormal.

Throughout life, maximum development of inherent capacity is dependent upon the alterations or limitations imposed upon the individual by environmental influences. At any one time the individual's placement upon the slope of biological time extending from conception to death, is determined by his inherent capacity for growth and development as altered by the restrictions superimposed upon it from external sources: materials or organisms taken (or not taken) into the body, actual bodily damage, and the restrictions of physical or mental activity. While we are not able to place definite, accurate values upon these influences, we can assess the condition of an individual at any one time, in relation to accepted qualifications of normality, by comprehensive examinations. By repeated measurements and observations under controlled conditions favorable to growth and development, an estimation may be made of his progress in biological time, though we cannot determine the relation between the actual changes and the capacity for change.

It is evident that the attainment of maximum capacity toward maturity requires growth and development during optimal nutritional conditions. Without measurements of capacity, these maximums cannot be determined, but, if we determine that an individual has no demonstrable abnormality and conforms to the standards set by comparable members of his species, we must accept his previous growth, development and nutritional status as having been within normal bounds. If we can certify, according to the best available criteria, that the individual's nutritional status is within the average range, we may expect it to remain within these limits under improved conditions of dietary and environment. We must assume that results from investigation of such an individual's biological processes represent the functioning of a human body within the range of normal variation.

When man does submit to investigation, heterogeneous and unstandardized subjects must be dealt with, for all the functions of life are remote consequences of heredity, previous environment, health, and care. The late T. Wingate Todd, cogently stated:[319] "The adult physical pattern is the outcome of growth along lines determined by heredity but enhanced, dwarfed, warped or mutilated in its expression by the influence of environment in the adventure of life." The fact that food supply may enhance or distort the potentialities conferred by heredity, prompted Sir Frederick Gowland Hopkins[147] to emphasize that "nurture may assist nature."[146] For this reason, in all studies of normal physiological functioning it is essential to select subjects who seem to possess maximal innate capabilities for physical and mental health, nutritional stability, adequate body development, well filled bodily reserves of chemical substances and temperaments amenable to control.

It is important, too, that the normal subject cooperates in all procedures and observations with enthusiasm, for emotional stress may, in itself, disrupt natural chemicophysiological functioning. As a rule it is best to try out each possible subject with a few simple tests to determine his qualifications for, and adaptability to, intensive training in experimental procedures and techniques. This avoids large expenditures of time, effort and money in collecting unreliable data.

Especially in a study of nutrition and chemical growth, it is essential that the subjects be carefully selected and the investigation initiated at an early age, in order that the maximum span of growth may be covered. Every effort should be bent to making each of the individual studies under analogous conditions and at specific and regular intervals. Each study should be continued as long as possible, in order to determine day-to-day, month-to-month, and season-to-season variations, unless extended observation is not compatible with unhampered physiological functioning and social development.

In a survey of nutrition and growth it is desirable to encompass individuals of both sexes, at frequent stages of ageing, so that the inherent variations at different age levels may be discovered,[38] however, one must not be over-zealous and attempt more experimental work than can be done with equanimity of both subjects and laboratory staff. Precision of method and accuracy of data are the first consideration, rather than numbers of subjects, variety or scope, and quantities of determinations. The knowledge gained and interpretations evolved from the results of investigation can be only as valuable as the reliability of the data obtained.

Equal consideration should be given to emotional and physiological factors, for in man they are deeply intertwined and each is directly influenced by the other. With children, as with adults, unvarying habits of hygiene, simply prepared foods served with regularity in an attractive and tasty manner amid comfortable surroundings, sharpen the appetite and promote better digestion and food utilization. Regular periods of sleep in comfortable single beds and well-ventilated, quiet sleeping quarters provide for growth and well being and reduce the nervous tension, wear and tear on the body, and therefore, fatigue. A generous amount of healthful exercise in the fresh air and sunshine promotes better circulation of the blood and nourishment of tissues. Wholesome companionship and constructive entertainment in all mental and physical activity stimulates an interest in the world about. Sympathetic understanding and security with a minimum of disruptions induce joy and happiness in living; pleasant and attractive surroundings have their aesthetic effect. All of these factors contribute to the interest and well being of the subjects and the successful organization and conclusion of the study.

The sole means of determining total nutritive requirement of the vital functions of a living body is the metabolic balance, in which are determined the actual amounts of nitrogen, calcium, phosphorus, and other equally essential dietary constituents retained in the body. This procedure includes quantitative chemical analyses for components of all food, urine, and feces; the difference between the ingesta and the egesta, over a definite period of time, is the quantity apparently retained in the body for its physiological activities. The mineral metabolic balance, so-called, is not a true balance, inasmuch as losses through the skin are unmeasurable over long periods of time or under usual environmental conditions. Tears, sweat, oral and nasal secretions constitute an additional potential source of mineral losses, mainly of chlorine, sodium, potassium and sulfur. From the relatively more accurate retention values for calcium, phosphorus and magnesium, the excretion of chlorine, sodium and potassium through the skin can be approximated.

Although a metabolic balance indicates the retention of an element by the body, knowledge of the amount of a single food substance assimilated is of little value in estimating the reasons for, or results of, the retention of that substance. The greater the number of dietary components determined, the greater the knowledge obtained about the relative proportions of the different substances being incorporated into the body, and the greater the opportunity for determining the controlling factors in the mechanisms by which the materials are being used. It is possible to predict, from a knowledge of the amounts of sodium, potassium and chloride retained in a given time, the distribution of body water between its two constituent forms, extracellular and intracellular water; from the amount or relative proportions of calcium and phosphorus stored, the bone or hard tissue formed can be estimated; from the amounts and proportions of nitrogen, sulfur, phosphorus and potassium retained, we can estimate the relative amount of soft tissues being formed. As a matter of fact, from a knowledge of the retention of positive and negative minerals, it is possible to determine with considerable accuracy whether the physiological tendency is in the direction of soft or hard tissue formation, but, with the present limitations of scientific information, it is not possible to hazard a guess as to which parts or organs of the body are being affected at any time.

A detailed study of the paths of excretion provides considerable information concerning the utilization of various chemical elements and serves as an integral part of a study of nutrition. Determination of the distribution of the nitrogen and sulfur components in the urine, the fat constituents in the feces, and the complex carbohydrates in the foods, as well as their disappearance in passage through the digestive tract, together with an acquaintance with gastrointestinal type, rate and motility—all these contribute to the total knowledge of nutrition, growth and metabolism. Supplementary observations—balances for iron, copper, nickel, manganese and zinc; the mineral and lipid distributions of the blood cells and plasma; basal metabolic rates; anthropometric measurements; roentgenograms to determine skeletal maturity, density of bony centers and gastrointestinal function; periodic observations of dental conditions—these also aid in the evaluation of physiological status and activities. The delineation of chemical growth would not be complete without accessory knowledge of emotional and intellectual status, inasmuch as effective functioning of the organism as a unit depends upon the symmetry of all the developmental processes.

The increasing numbers of chemical, physiological and psychological tests that recently have been applied in the evaluation of nutrition and growth have been made possible through the development of new techniques in the fundamental and applied sciences. As a result of these new tools and approaches being directed towards a study of the individual while he is evolving into an adult (some may choose to consider this transformation of changing structure and function as growth, others as ageing), the most impelling problem today resides in the discovery of the relatedness to one another of these and other chemicophysiological tests in describing the structure and progression of the organism as a whole. When we realize that only recently have the rudiments of this essential knowledge been obtained, it is not too much to expect that with each successive addition of facts we will become better qualified to contrive experiments for the quantitative measurement of the chemicophysiological changes which occur with progress in structure and function during ageing of the human body.

In planning a balance study, the investigator must choose the methods and procedures which will provide data most pertinent to the objectives of the research. Restrictions owing to limitations of staff, equipment, facilities and finance are frequently encountered and these considerations should be met in the planning, rather than after initiating the routine of investigation. Proper allowance must be made for accidents and breakdowns of equipment in contemplating an extended program. Also, proper allocation of the time available for the study must recognize that compilation and interpretation of the results frequently requires more time than their accumulation. The workers involved should not be taxed with responsibilities great enough to produce the possibility of errors resulting from fatigue.

The novice in balance investigation, particularly the graduate student, is prone to overestimate the productive possibilities of his time and resources. Some methods and procedures have variable value and reliability, determined by the conditions under which they are used and sometimes by the length of time over which they are applied. All the foregoing factors, those of climate, season, and other attributes of environment, together with those of age, sex and individual characteristics of the subjects may determine the choice of experimental techniques. Investigation of morbid subjects requires preciseness in the selection of experimental procedures which will provide information of maximal value in the diagnosis, treatment, or prevention of the dyscrasia involved. One fact cannot be too strongly emphasized; a few data from coordinated procedures carefully executed, meticulously recorded, capably interpreted, and clearly presented, are of far greater value than a larger number of less reliable figures.

In this volume, the material used in the illustrations and all of the data presented (except for a few comparisons which are specifically noted) have been assembled from the results of studies conducted by the Laboratory. The subjects of all the studies were healthy individuals selected by the criteria set forth in following chapters and the values were obtained by the techniques and methods presented.

Nomenclature

The impossibility of choosing terminology which is accurate and has the same connotation to all readers, demands that the author of a scientific treatise clearly define the limits within which the reader should interpret the meaning of controversial terms. There is little agreement upon nomenclature among the scientists investigating the question of "child development" from different angles of approach. Even the terms "growth" and "development" have not acquired definition and differentiation acceptable to the entire group. The obstacles hampering attainment of clear, concise, and non-conflicting terminology are increased by the gaps in our knowledge of actual occurrences within living cells, tissues and organisms.

In this volume we have not attached greater significance to a term than is justified by its etymological meaning and general usage. Our efforts have been directed toward selection of words whose widely accepted meanings and connotations would convey, exactly, the thoughts of the author. We have not intended that any implication beyond that recognized by *Webster's New International Dictionary*, be attributed to a term or phrase, however, to avoid ambiguity in presenting the material, specific definition of some terms has seemed advisable.

We have restricted *growth* to the meaning *increase in size.* Designations such as *negative growth* have been eliminated in favor of exact expressions. We have used *development* to indicate progress in the specialization, organization, or differentiation of tissues, function, or the entire organism. For the purpose of this presentation, *childhood* refers to the years between the ages of four and thirteen.

Because of its prevalence in the literature we have used *plane of nutrition* to mean level of caloric intake. In referring to the nutritional performance level of individuals we have applied *nutritional status,* which encompasses both level and efficiency by which food materials, or nutriments, are utilized in the nutritive processes involved in nutrition.

Metabolic balance has been applied to the procedure of determining quantitative intake of a food constituent; the corresponding, quantitative outgo of that constituent in feces and urine; and the calculation of the difference between intake and outgo. This difference has been arbitrarily designated by the simple terms *retention* (when intake is larger than outgo) and *loss* (when outgo is larger than intake). The difference between the heat of combustion of food intake and the heat of combustion of the outgo (urine plus feces) we have designated as physiologic fuel value, utilizable energy, metabolizable energy, available energy or fuel value.

Frequently the terms *retention, absorption, assimilation, storage, laid down, deposition, utilization, accumulation* and *accretion* are used interchangeably. The word *apparent* has been attached to most of these terms at one time or another by various authors, in attempts to be more specific. We have tried to pursue a course directed toward simplicity, in the interest of accuracy without confusion, and at the risk of accusations of being arbitrary. We have distinguished between: *retention,* the arbitrary designation of the balance between intake and outgo in urine and feces; *absorption,* which is concerned only with the quantity of substance taken into the body and, when applied to actual values, indicates the difference between intake and fecal excretion; *deposition* and *laid down,* which connote actual incorporation into body tissue; *storage,* which implies deposition as reserve, for future use; and, *utilization,* which indicates active participation in body activities. *Assimilation* encompasses absorption, retention, and utilization. *Accumulation* and *accretion* refer to the increments in deposition or storage over a period of time.

Minerals is used as a general term to refer to inorganic chemical elements which are involved in body processes. When possible we have avoided referring to the so-called "trace elements" or "trace minerals," for the retentions of some of these elements (zinc, copper) are frequently larger than the retentions of iron, which is not usually included in the group. Occasionally, the expression "heavy metals" has been applied to the elements: copper, iron, manganese, nickel and zinc.

We have adopted many of the terms used by Shohl in *Mineral Metabolism*.[312] *Positive minerals* are calcium, sodium, potassium, and magnesium. *Negative minerals* are phosphorus, chlorine and sulfur. *Total positive minerals* and *total negative minerals* refer to the sum of the minerals in their respective groups. In discussing electroneutrality, the maintenance of ionic equilibrium in body tissue, positive and negative minerals are expressed in equivalents (eq.) or milliequivalents (meq.). Whether the excess value represents positive or negative minerals is sometimes indicated by a positive or negative symbol placed after, and superior to, the figure. (In making this designation we have extended the customary method of indicating an ion radical (H^+Cl^-) to figures.) The excess mineral balance (sometimes called acid-base balance) is obtained as the difference between the excess of positive or negative minerals in the food intake and the excess of positive or negative minerals in the outgo (urine plus feces).

The total of positive and negative minerals is the ash value for the food intake and the total mineral value for the urine and for the feces. The relationship between total positive (base-forming) and total negative (acid-forming) minerals in the food intake, in the urine and in the feces, is indicated by the *excess* of positive over negative minerals or of negative over positive minerals. To designate this excess for the food intake the terms *alkaline-ash value* and *acid-ash value* have been used. For urine and for feces the excess of positive over negative minerals, or of negative over positive minerals, is given as excess of positive or negative minerals.

CHAPTER II

GENERAL CONSIDERATIONS IN METABOLIC BALANCE STUDIES

FROM conception to death, health is primarily dependent upon food and nutrition. The adequacy of nutrition is a major determinant of growth and development *in utero,* during infancy and childhood. Without adequate nutrition, health is impossible and the body is easy prey to accident and disease. Inadequate nutrition in adulthood also may have repercussions in the endowment of the succeeding generation.[317]

We have emphasized that the campaign for the better nutrition of children should have its inauguration with the mother during pregnancy, for at that time the child is wholly dependent on the maternal body for sustenance and for the rich or lean physical endowment with which it begins postnatal existence.[219] The chemical elements that are furnished the embryo are derived from the maternal diet or tissues. During lactation, both mother and child are co-recipients of the benefits derived from adequate maternal nutrition or the deleterious effects of unsatisfactory nutrition. After the nursing period, the child becomes an independent entity but may be influenced by untoward environmental factors, depending upon the adequacy of his nutritional endowment, on the one hand, and upon the intensity of unfavorable influence on the other. Parents deserve the best scientific advice and knowledge to aid them in rearing healthy children. The normal, growing child is emphasized in this volume, but the factors which influence the growing child are reflected in adulthood and problems discussed in relation to growing children may be equally pertinent in the lives of infants and adults.

The development and maintenance of healthy body structure depends upon supplies of many food substances. Consideration must be given to the kinds and amounts of protein and fat in the diet, to the deficiencies or excesses of different minerals, to the presence of vitamins, and to the types of carbohydrates ingested. Insufficient food, or an unbalanced food intake, may result in faulty metabolism which may be reflected in a debilitated state of nutrition, although growth may *seem* to be taking place in accordance with the accepted standards. It is known that the daily requirement of many individually indispensable food substances is increased during the maturation of physiologic processes in growth and adolescence.

Inherent Physiological Characteristics

Every individual has a characteristic physiological capacity to utilize and store chemical elements[120] from ingested foodstuffs, predetermined by heredity and difficult to evaluate, for it may be modified by life's experiences. This factor must be carefully regarded in the interpretation of experimental observations. A child may be destined to be tall or short; to progress rapidly or slowly through physiological time, which will determine whether maturity will be attained at an early or late chronological age; or, to possess a characteristic endocrine system[109, 193] or organization of other tissues which will control the type of chemical growth taking place at all times. An individual may develop a buccal apparatus[233] which modifies mastication and gastronomy or possess a gastrointestinal tract longer or shorter than his fellows, of more or less motility, and subject to irregular amounts and concentrations of digestive secretions. All of these factors influence the metabolic performance of the individual.

Environment and habit may play important roles in restricting or encouraging physical development to the limit of inherent capacity. Physical activity, available foods, eating and elimination habits, and mental state may alter intestinal lag and other physiological responses. All of these factors have a significant bearing upon the absorption and retention of food given at any time, whether the subject is a growing child or an adult. While we agree with Sherman,[307] who has pointed out that "the recognition that our control of food and other environmental factors can enhance the potentialities conferred by heredity is highly important," it is prudent to give the above individual characteristics equal consideration when interpreting metabolic data, especially in relation to growth and the efficacy of different kinds of food materials.

The child who is advancing in physiological age more rapidly than most children of the same chronological age, may evidence periods of greatly accelerated skeletal growth by unusually large deposition of bone-building materials. At intervals during childhood an individual may be losing weight, yet be normal by every known clinical criterion and be "growing," i.e., actually increasing his mass of living tissue, by retention of chemical elements from the food intake.

Individual Variation

Individual human beings are of many types, some constructed upon a framework of large, massive bones while others possess more delicate skeletal structure. Even in childhood certain individuals tend to be heavily muscled, others fatten easily, and extreme, yet normal, variations in characteristics of human construction and function may be found among individuals. One subject who cooperated in the studies of this laboratory was assigned a skeletal maturity (differential skeletal maturation) of 5 years, 8 months, from roentgenograms taken at the chronological age of 4 years, 10 months. According to anthropometric measurements and clinical examination, the child conformed with the standards for his chronological age, even though he was a year advanced in skeletal maturity. In Figure 1, the calcium retention of this child (A) over 45 consecutive days is compared with that of another child (B) of similar chronological age but not advanced in skeletal maturity, while the children were consuming similar diets containing slightly less than one gram of calcium per day and living in the same environment. Since 99 per cent of the calcium retained in the body is laid down in skeletal tissue,[309] it is reasonable to assume that the greater retention of calcium maintained by subject A (averaging 469 mg./day while B averaged 264 mg.) was in response to body needs to keep pace with accelerated physiological age.

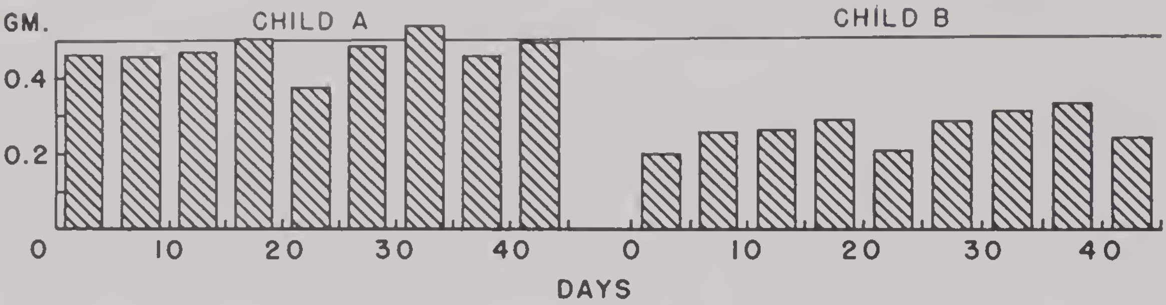

FIGURE 1. Calcium retention by two children of similar chronological age while ingesting diets of identical calcium content.

During the course of metabolic balance studies and growth observations over the past twenty years we have become more and more impressed with the wide physiological variations which occur, and we have learned to respect these constant stimulators of caution in the interpretation of data. Chemical fluctuations are evident among individuals of the same age, body size, and living under the same environmental conditions, who were observed for several months at a time. Each individual seems to have certain characteristic patterns of physiological performance, in spite of the adaption and integration that becomes necessary in the adventure of life. The need for specific knowledge of the degree of variation that may be expected in a healthy individual living under highly standardized conditions is evident and it is exceedingly important to obtain some concept of the chemical changes that occur incident to growth and development in childhood.

Nutritional Status and Physiological Activity

Although Rubner, in 1885, showed that nutritional status of the individual markedly influenced the amount of, and rate at which nutriments are used by the body, not enough consideration has been given this important fact in evaluating metabolic data. In interpreting Rubner's findings, Lusk[206] states that according to these laws adult cells which have been depleted of their protein may gradually improve their nutritive condition until they reach an optimum, at which point they lose their power to attach additional protein. The plane of nutrition of an individual may determine the extent to which certain elements are retained or rejected by the body. The malnourished individual who has been *underfed* will tend to store large amounts of nutriments when his diet is improved, while the *misfed* actually may be inhibited from using certain materials until chemical adjustments have taken place within the tissues to such a degree as to permit retention. There is provision in the tissues for deposit of certain elements[6] in excess of current needs, a storage or bodily reserve. Differences in human beings' metabolic response owing to their nutritional status have been pointed out by Clark,[48] Stearns, *et al.*,[328–330] Wang, *et al.*,[364–367] Daniels, *et al.*,[62] and, in a young primipara, from this laboratory.[156] Similar observations have been made on animals [91,309]

The effects of nutritional status are illustrated by the average daily retentions of nitrogen, calcium, and phosphorus of two comparable groups of boys, ages four to six years. Each group consisted of three subjects and the diets of the two groups were approximately equivalent (Table 1). The children in group A were allowed the conventional adjustment period of 10 days on the experimental dietary; those in group B received a quart of milk per day, superimposed upon a generous mixed diet, for three months before initiation of the study. The data demonstrate lower average daily retentions by the children whose body stores were augmented during the long prefeeding period, thereby improving their nutritional status prior to initiation of the study regime and reducing their requirements during the investigation. The values demonstrate the wisdom of a rather lengthy prefeeding period, for experimental subjects, to permit saturation of the tissues, before attempting to use them to evaluate the nutrient value of any one food or dietary regimen to the normal individual.

TABLE 1

INFLUENCE OF LENGTH OF PREFEEDING PERIOD UPON MINERAL RETENTION

Group	AVERAGE DAILY RETENTION*				
	Nitrogen	Calcium	Phosphorus	Minerals	
				Positive†	Negative‡
	mg.	mg.	mg.	meq.	meq.
A§	867	473	438	68	54
B#	280	103	61	15	8

* During 55 consecutive days.
† Sum of calcium, magnesium, potassium and sodium.
‡ Sum of chlorine, phosphorus and sulfur.
§ Period for which data are given followed conventional ten-day adjustment period.
# Period for which data are given followed three-month adjustment period upon a generous mixed diet which included one quart of milk per day.

The results shown in Table 1 are in accord with those of Fairbanks and Mitchell,[91] who have shown with rats that "differences in the degree of saturation of the skeletal tissues with respect to calcium, brought about by previous subsistence upon diets differing in their contents of this element, produce inequalities in the retention of calcium under uniform conditions of calcium nutrition, such that low saturation is associated with subsequent high retention of calcium."

Sufficient food may be made available in the digestive tract but there are physiological factors within the organism that may influence its utilization. It is necessary to go beyond the conventional procedure of determining retentions of calcium, magnesium and phosphorus in evaluating all the qualities of a foodstuff and to obtain more precise understanding of chemical interrelationships in physiological activity, in health and growth.

If phosphorus is fed in excessive amounts calcium in the feces is augmented, for more insoluble phosphate is formed. Similarly, a greater intake of calcium leads to a greater fecal outgo of phosphate.[255] Hydrochloric acid or any substance that produces acidity in the intestinal tract exerts a favorable influence on the calcium absorption, because the acidity permits transformation of phosphate, carbonate and soaps of calcium into more soluble compounds and permits elimination of more fatty acid in the free acid state.[33,115,165,342] Fatty acids, which are formed during the digestion of neutral fat, seem to create favorable conditions for calcium absorption. They do so by acidifying the intestinal contents and by combining with calcium to form calcium soaps which may be dissolved and absorbed through the action of the bile acids.[214] Part of the confusion in regard to the interrelationship of fat and calcium absorption may arise from the type of fat used in the dietaries of various investigations.[28] Fats containing a large proportion of fatty acids of lower molecular weight (such as milk fat) in contrast to those containing fatty acids of higher molecular weights (lard), may lower the *pH* of the alimentary tract while the acidity of the latter is negligible. Alkaline earths produce opposite effects according to some investigators,[64] but none according to others.

No two natural foods are exactly alike; their caloric values may be identical while their carbohydrates vary in availability and the indigestible portions possess different physical characteristics;[158] their nitrogen values may be similar but their mineral contents unlike and the various minerals present in dissimilar proportions; the various vitamins may be present in effective proportions and the proportions of the different amino acids in the proteins be so different that one food produces nutritive success and the other, failure. Even the type and quantity of fiber in the diet may, by influencing the passage of material through the gastrointestinal tract, or by exerting a physiological effect upon the secretions of the gut itself, affect the digestion and absorption of all food constituents. Positive knowledge of the physiologic effects produced by dietary alterations is needed.[222]

We have been able to illustrate the subtle influences of simple dietary changes based upon the premise, current in dietetic practice, that one food may be substituted for another on the basis of a single food principle without regard for equally important, coexisting dietary requirements. The effect of substituting 100 gm. of banana for 10 gm. of white bread and 20 gm. of breakfast food in the mixed diets of five children, on the basis of the approximately equal caloric values of the banana and the cereal, is shown in Table 2.

TABLE 2

CHANGES IN AVERAGE DAILY RETENTIONS BY FIVE CHILDREN CONSEQUENT TO MINOR FOOD SUBSTITUTION IN DIETS*

	Pre-experimental (170 days)		Experimental (175 days)	
	Mean	SEm†	Mean	SEm†
Energy intake (Cal.)‡	1685	11	1699	11
Physiologic fuel value (Cal.)§	1554	10	1571	10
Fat intake (gm.)	65.2	1.4	67.0	1.3
Fat retention (gm.)‖	63.5	1.4	65.2	1.2
Nitrogen intake (gm.)	9.84	0.07	9.67	0.07
Nitrogen retention (gm.)	0.46	0.06	0.54	0.05
Food intake				
Ash value (meq.)	390.0	2.4	392.7	1.2
Positive minerals (meq.)#	201.0	1.0	207.2	1.8
Negative minerals (meq.)+	189.0	1.4	185.5	1.8
Alkaline-ash value (meq.)	12.0	0.4	21.7	0.8
Urine				
Total positive and negative minerals (meq.)	288.6	3.3	289.0	2.5
Positive minerals (meq.)#	137.7	1.8	140.9	1.3
Negative minerals (meq.)+	150.9	1.6	148.1	1.4
Excess of negative minerals (meq.)	13.2	0.9	7.2	1.0
Feces				
Total positive and negative minerals (meq.)	73.7	1.8	71.3	1.7
Positive minerals (meq.)#	49.0	1.0	48.5	1.1
Negative minerals (meq.)+	24.7	1.0	22.8	0.7
Excess of positive minerals (meq.)	24.3	0.9	25.7	0.9
Retention				
Total positive and negative minerals	27.7	1.8	32.4	1.8
Positive minerals (meq.)#	14.3	1.0	17.8	1.1
Negative minerals (meq.)+	13.4	1.3	14.6	1.1
Excess of positive minerals (meq.)	0.9	1.3	3.2	1.4

* During the experimental period 100 gm. of banana were substituted for 10 gm. white bread and 20 gm. cereal.

† SEm represents the standard error of the mean.

‡ Gross calories determined as heat of combustion.

§ Energy intake minus heat of combustion of urine and feces.

‖ Difference between fat intake and fat excretion in feces has been arbitrarily designated as fat retention.

Positive minerals are calcium, magnesium, potassium and sodium.

+ Negative minerals are chlorine, phosphorus and sulfur. Valences of 1.8 for phosphorus and 2 for sulfur were used in calculations.

From the responses of the children there are factors other than plane of nutrition which influence the retention of minerals. Moreover, the data demonstrate that selective retentions occur, regardless of caloric value, intake of nitrogen, positive and negative minerals or the acid- or alkaline-ash value of the diet. Fat intake and retention were both increased by the substitution in the diets; nitrogen intake was slightly lowered but the average retention increased. The intake of positive minerals was increased and of negative minerals, decreased. Parallel changes occurred in the urine but excretion of both positive and negative minerals in the feces were reduced.

Explanations for the type of response that a child gives to a specific dietary change must be sought in the effects of altering the interrelationship of individual mineral constituents or other components of the diet at the same time intakes of nitrogen, positive and negative minerals are varied. The proportions of the individual positive and negative mineral elements in the diet may have more importance in metabolism during growth than the total or excess quantities of the two groups of elements. Mitchell and coworkers[311] have shown that the constituents of vegetables tend to depress the utilization of the calcium of other foods with which they were fed. They suggest that the indigestible carbohydrates may impair digestion and absorption from the intestinal tract by rendering the calcium compounds in vegetables less accessible to the solvent action of the digestive juices.

Individuals vary in their ability to utilize milk and other common foods. Outhouse, Mitchell, and their associates[179,331] have emphasized the fact that it is as necessary to know how well a person utilizes the calcium of the food which he eats as it is to know the actual calcium content of those foods. In an attempt to determine the degree of calcium utilization from milk, by pre-school children, these authors have computed the "percentage utilization," or percentage retention, of the *increment* in calcium intake.* They found that pre-school children were able to utilize only one-fifth to one-fourth of the calcium in milk, on the average 19 per cent of the calcium in the milk was retained, but there were variations among the different children in their ability to use the calcium of milk. We have applied this method of deter-

---

* $$\frac{\text{Retention for period } X - \text{retention for period } Y}{\text{Intake for period } X - \text{Intake for period } Y} \times 100 = \text{per cent utilization}$$

where X represents a period of high intake and Y signifies one of lower intake.

mining the ability of children to utilize calcium from milk to the results obtained with five children during an experimental period of 140 days in which the only change in dietary was an increase in milk consumed. During the pre-experimental period of 150 days, the average daily calcium intake per child was 1090 mg. In the experimental period the calcium intake was augmented by 898 mg. per day. The increased calcium intake provided by milk, resulted in an increase of 363 mg. in the average daily retention, 40 per cent of the increase in calcium intake. Since the subjects were all in positive calcium balance during the pre-experimental period, the conditions set forth by the Illinois investigators were satisfied by the study, but, instead of averaging 19 per cent retention of the calcium added to the diets in milk, our five children averaged 40 per cent. One subject, during a twenty-day period immediately following the experimental period in which the milk intake was increased, was returned to the same daily food intake as in the pre-experimental period. The child's average daily retention of calcium for the 20 days was reduced by 254 mg.

During investigation of nutrition and chemical growth, normal individuals were found, who, over considerable periods of time, were losing weight and at the same time storing nitrogen and all of the electropositive and electronegative minerals (Figure 2). More information is needed concerning physiological activity during normal growth of different magnitudes, during highly controlled metabolic procedures. It is important to bear in mind that ideal growth may not, necessarily, be the most rapid growth but may be the growth-rate induced by the plan of feeding which yields the best results for the life cycle as a whole.[374–376]

Seasonal Changes

The seasons, environmental temperature and humidity are extrinsic factors that are alleged to affect the physiological processes of the body.[72,265] Ultraviolet irradiation from sunshine[133,346] enhances mineral retention, especially of calcium and phosphorus, when the diet is inadequate in vitamin D. The amount of ultraviolet light available to man not only depends upon the season of the year and the time of day, but upon the freedom of the atmosphere from dust particles. Certainly, in metabolic studies of growing children, it is the part of wisdom to conduct comparable studies during the same season of the year.

Definite determination of the influence of sunlight upon body function will require studies during the summer months, however, any attempt to carry on metabolic balance investigation during the hot season is hampered by additional difficulties in the control of subjects, environment and analyses. Important among the difficulties presented by exceptionally warm weather are the sensible perspiration losses from the body and psychological interference on the part of the subjects. Season is known to influence blood calcium, phosphorus and lipids[150] but seasonal alterations in the body and in its functioning deserve more intensive study, under specially planned conditions which will meet the requirements of accurate and definite interpretation.

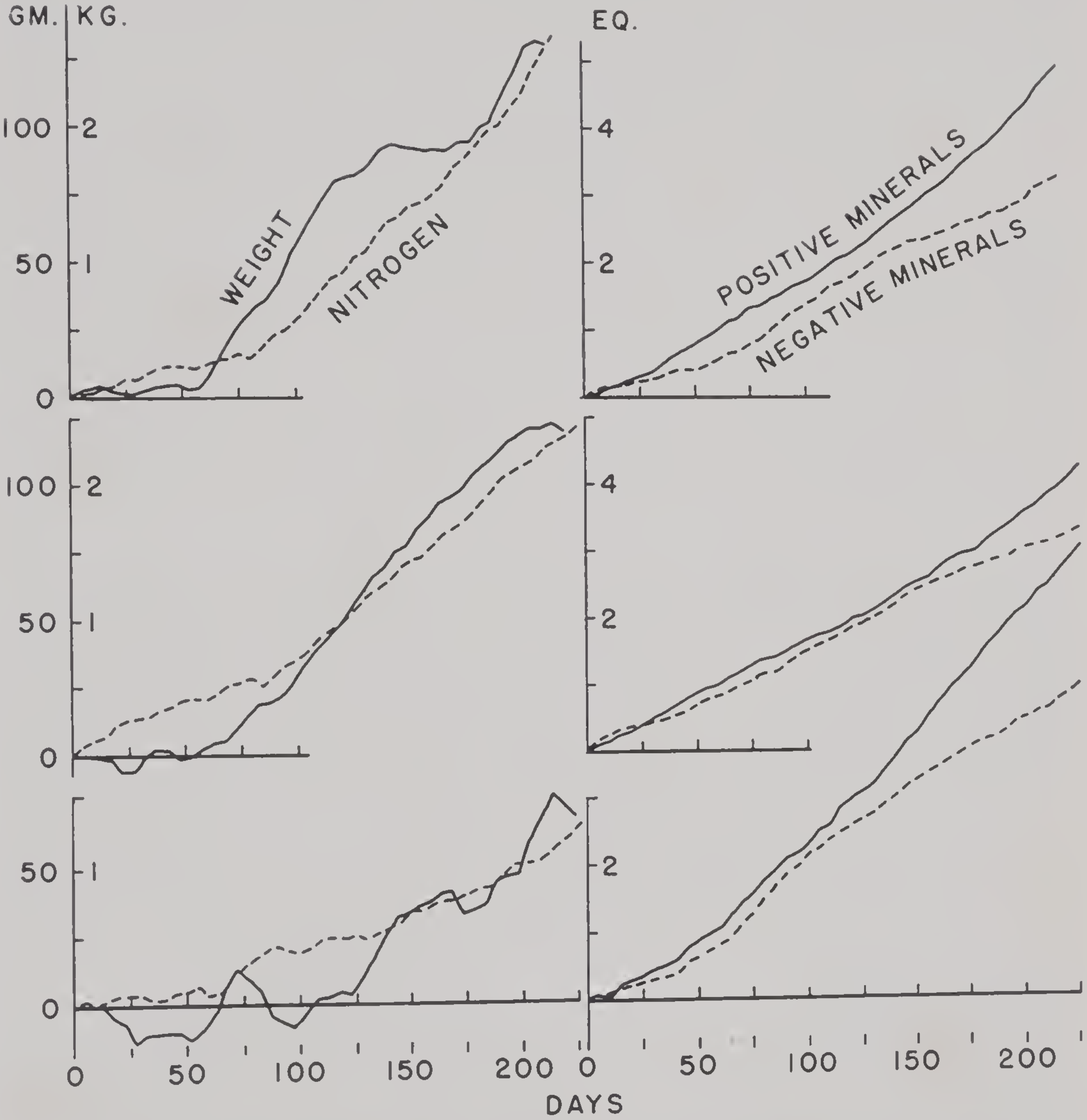

Figure 2. Gain in weight and accumulation of nitrogen and positive and negative minerals over 225 consecutive days by three children.

Diurnal Physicochemical Changes

The body experiences diurnal variations, chemical and physiological, which manifest themselves in various ways. That urine excreted at night is more concentrated than day urine is illustrated by the analyses of the urine of a four year old child, in Table 3.

TABLE 3

URINE ANALYSES FOR A PRESCHOOL CHILD

	Urine collected during night*		Urine collected during day†	
Volume (ml.)	369		764	
Specific gravity (corrected to 15° C.)	1.0183		1.0147	
pH	6.2		6.6	
	Content		Content	
	per 12 hours	per liter	per 12 hours	per liter
Total solids (gm.)	17.6	47.7	29.2	38.2
Total nitrogen (mg.)	2959	8019	3095	4051
Urea (mg.)	2749	7450	2940	3848
Ammonia (mg.)	96.7	262	111.0	145
Creatinine (mg.)	38.4	104	48.2	63
Creatine (mg.)	24.7	67	52.5	69
Uric acid (mg.)	25.1	68	45.3	59

* 7:00 P.M. to 7:00 A.M.
† 7:00 A.M. to 7:00 P.M.

Figure 3 contains notable illustrations of the diurnal physiologic variations in urinary *pH* for a group of normal children,

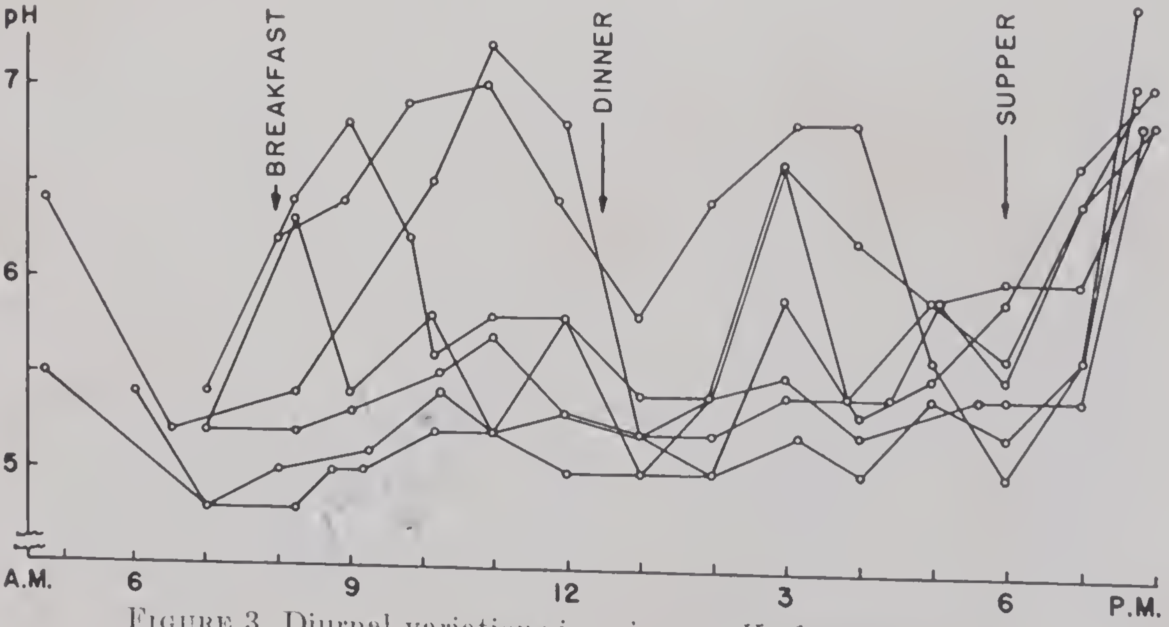

FIGURE 3. Diurnal variations in urinary *pH* of seven children.

four boys and three girls, aged four to ten years, while ingesting the same foods and living within the same environment. The pH values of the urine not only fluctuated throughout the day for the group but the individuals varied even though they consumed the same food and lived in the same environment.

Recently, Hastings and Eisels[126] have presented experimental evidence to show that the acid-base balance [equilibrium] of the blood of a normal adult at rest varies in a regular, not random, manner during the 24-hour period. They found a rise in plasma bicarbonate associated with ingestion of food. An increase of carbon dioxide tension of the blood was associated with sleep. The number of white blood cells is subject to diurnal changes, also, as is shown in Figure 4 by data obtained with two women, under standardized routine and refined techniques of blood sampling and counting.[177] There are indications that these fluctuations may be characteristic of the individual.

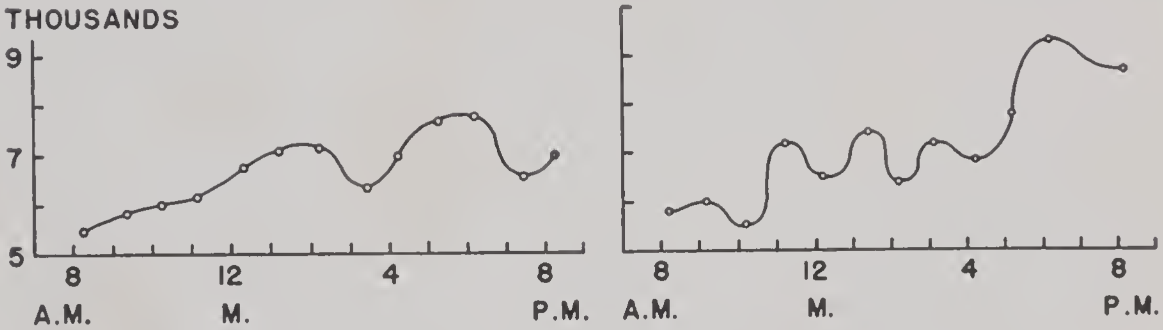

FIGURE 4. Diurnal changes in the white blood cell counts of two women.

Physical and Emotional Activity

Physical exertion operates to influence metabolism, even with children of similar ages while living under the same environmental conditions and experiencing the same regimen. One child may cover only two miles, according to a pedometer reading, while another covers seven miles in the same interval of time. Although this method of measuring activity leaves much to be desired in the way of specificity, it does evidence the large quantitative differences in the physical activity of young school children. Oxygen consumption and mineral and water excretion through the skin are both affected by the intensity of physical activity; in turn, these are reflected in the respiratory, circulatory[170] and chemical metabolism. Schlutz, *et al.*,[291] studied the response of children to exercises, in an attempt to relate physical capacity to the changes in nutritive state, and used a great many chemical and physiological tests which offer fruitful fields for further investigation.

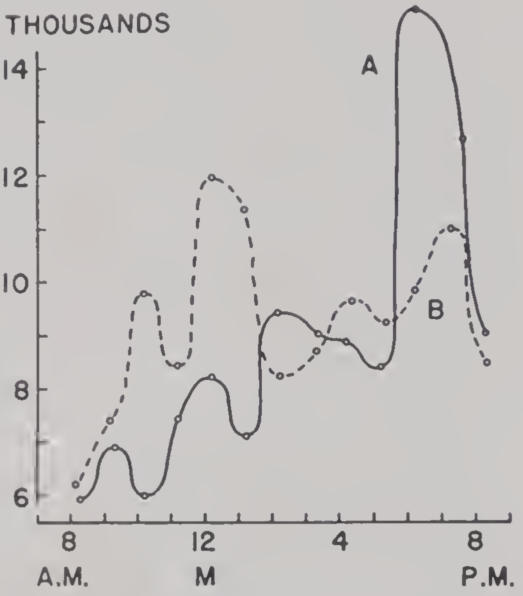

FIGURE 5. White blood cell counts of one woman upon two successive days, A and B.

Emotional disturbances such as fear, anger and pain are known to affect the physiological activities of the body.[45,70] These may be demonstrated in various metabolic channels; by more frequent or suppressed urination or defecation, by retardation or cessation of milk flow, by change in blood pressure, by an increased number of white blood cells, by increased perspiration, and by flushing or chilling. Figure 5 illustrates the effect of anxiety on the white blood cell count of a woman who, during an hourly hematological study over a 13-hour period, experienced on one occasion (Curve A) a period of extreme anxiety, from 5:00 P.M. to 6:00 P.M.

During a carefully conducted study, 1562 white blood cell counts on 540 healthy infants gave further evidence of the effect of emotion upon the leucocyte counts. In determining normal values for infants statistical comparison of the averages of counts made while infants were quiescent, crying, restless and crying vigorously, warranted deleting as abnormal the high counts made when the infants were restless or crying vigorously (Table 4). Ferris[96] has recently investigated the affects of fear, anger

TABLE 4

EFFECT OF EMOTION ON WHITE BLOOD CELL COUNTS

	Number of infants	White blood cell count	
		Means	Deviation
Emotional state			
Quiet	532	10,280	2080
Crying	52	10,200	3360
Restless	55	11,570	2940
Crying vigorously	92	11,460	4550

and disappointment on the differential blood counts of man and has supported our earlier observations (see pp. 30–33) that states of anxiety produce relatively marked lymphocytosis in healthy individuals. The greater the degree of emotion, the more marked is the relative lymphocytosis.

During roentgenological investigation of gastrointestinal response, a nine year old girl became disturbed prior to exposure of a series of roentgenograms—the films exposed during her anxiety showed wide variations from those taken after she became familiar with the procedure.

In a study of the mineral metabolism of pregnant women, one subject's mother died when the subject was in the 26th week of pregnancy (Figure 6). During the week of emotional disturbance, although the subject ate the usual amount of food, she showed a large negative calcium balance, accompanied by other physiological and chemical disturbances.

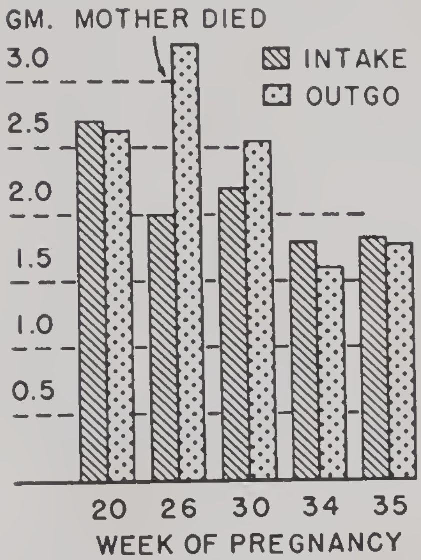

FIGURE 6. Negative calcium balance during emotional stress.

Gastrointestinal Activity

In any quest for information concerning the transformation of food into living tissue or body activity, the wide variations among different individuals' gastrointestinal responses are extremely important. Whether rate of digestion determines gastric and intestinal emptying times, or the speed of gastric and intestinal evacuation influences digestion, the mechanical reactions which occur in the tract influence the processes of digestion, absorption and assimilation—as do intestinal lag, enzymatic activity, intestinal surface exposed to the food residue, eating and elimination habits.

Type and motility of the alimentary canal have long been recognized as prime influences upon digestion and absorption. Many studies of the functions and activity of the gastrointestinal tract have been made in attempts to discover some of the effects of its physiological activity upon utilization of the food elements needed to maintain adequate body nutrition. Some have failed to contribute scientific knowledge, either because unsatisfactory methods have been used, or because the procedures have not been accurately controlled or carried on for sufficient time. Also concerned in the failure of such studies to yield pertinent information, is the fact that they included only a single phase of absorption or excretion of foods and in interpreting results, the investigators neglected other, equally important factors. The complex nature of the problem of absorption alone has been presented by Verzár.[357] Investigations have been carried out to determine the role of the nervous system; the optimal rate of passage of material through the gastrointestinal tract; and, the effect of bulk and the form in which feces should be excreted from the large intestine.

Metabolic balance studies do not permit exact evaluation of the functioning of the gastrointestinal tract because, in feces, those elements that are present as a result of incomplete digestion of food cannot be separated from those that have been absorbed from the upper intestine and excreted into the lower bowel. However, several types of individual determinations or combinations of several observations may be used to clarify the role of the gastrointestinal tract in nutrition. Among these are: the number of bowel movements per day (laxation rate); the time required for food to pass through the tract (measured by a marker ingested with the food); the evacuation time of the tract and its component parts (by roentgenograms); and the mass, consistency, and composition of the fecal material ejected from the rectum. The urinary excretion of ethereal sulfur may also be indicative of gastrointestinal function.

The average daily wet and dry weights of the feces of two boys during 55 consecutive days are shown in Figure 7. Wide variations from period to period are evident for each subject although their daily food intakes were identical in quality and quantity. Meanwhile, both children were in excellent health and had approximately the same number of bowel movements per day, the stools of the child who ejected the larger amounts of feces were bulky, and soft and unformed; those of the other boy were hard and the fecal units bore haustral markings.

Characteristics of the average daily fecal excretion of 7 children, 8 to 12 years old, during 55 consecutive days, are shown in Figure 8. Wide variations in the average daily intestinal excretion of the different subjects are evident, although their food intakes were of the same quality. A part of this variation may be attributed to the long night intervals during which defecations seldom occurred, while during the day there was stimulation and frequent opportunity for elimination. However, this condition does not account for the inconsistencies among the individuals which are evident from the average values (Figure 8).

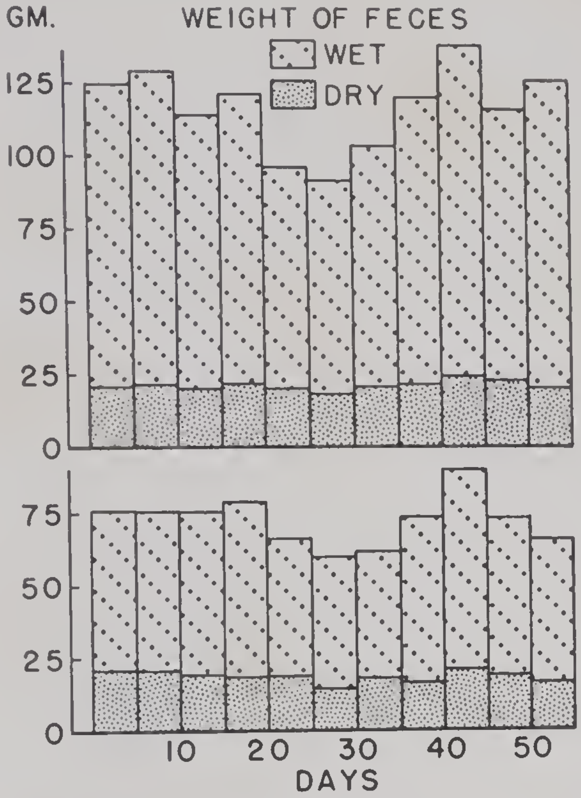

FIGURE 7. Wet and dry weights of feces of two boys during 55 consecutive days.

Observations in mineral metabolism have made it clear that the paths of excretion through the kidneys and intestine are closely related and that certain foods or conditions may cause a change in path of excretion from the urine to the feces, for example, an increased fecal output of calcium in the feces may be compensated by a decrease in output of calcium in the urine. The mineral elements which seem to be most susceptible to exchange between the urine and feces are calcium, phosphorus, sodium, potassium and chlorine.

Results show that the fermentation of cellulose and hemicellulose in the tract are closely related and that the disappearance of these from the intestinal tract of healthy children with regular habits of elimination is characteristic for each individual. Children whose laxation rates indicate a slow intestinal tract, digest more cellulose and hemicellulose than children with more rapid tracts. Children having a higher dry weight of feces and a lower output of cellulose and hemicellulose (indicating longer fecal retention and more extensive fermentation) show higher levels of urinary ethereal sulfate. This is another indication that the level of urinary ethereal sulfate may be related to fecal retention and intestinal putrifaction.

Our results indicate that an average of one bowel movement or more per day, with elimination in not more than 36 hours, yields a minimum absorption of toxic substances, judging from the ethereal sulfate excretion. The increased fermentation of the complex carbohydrates, a decreased water content of the feces, a decrease in number of bowel movements per day, or longer food passage time as children approach maturity may be associated with growth in length of the intestine. If this is true, age is an important factor in considering the normal laxation rate of children. The type of bacterial flora (fermentation or putrefaction types) present are also of importance and, in connection with other physiological observations, aids in evaluating metabolic data secured on individual subjects.

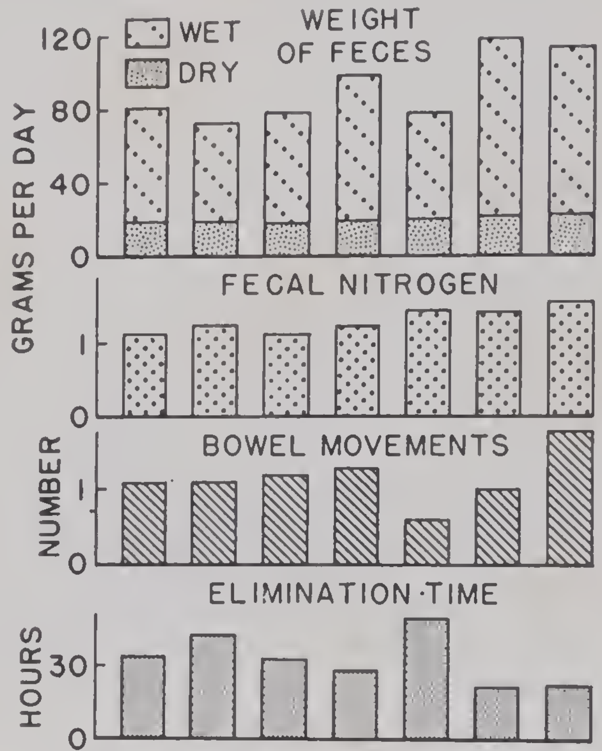

Figure 8. Characteristics of fecal excretion of seven children.

Formed Elements of the Blood

Coincident with procurement of newer knowledge of the chemical, physical, physiologic and psychologic processes of living organisms, have come determined efforts to understand the integration of these intricate forces in the human being, as he changes from a physiologically incomplete and unstable form to one that is mature in both structure and function. How this immaturity develops into the steady functioning of the normal adult, or perhaps fails to do so in the abnormal adult, is a field of investigation largely unexplored but yet has a vital bearing upon proper understanding of the young child in health and disease. Moreover, physiologic changes coincident with growth may be first portrayed by the components and structure of the blood (See, Chap. IX) and the composition of this fluid is a determining factor in the nutrition of the entire body.

Relations Among Formed Elements

The origins and functions of hemoglobin and the formed elements of blood, red blood cells, white blood cells and platelets are quite different. The quantity and structure of each constituent provides an index of the status of the tissues from which it is derived, and, in case of pathologic disturbance or in case of structural and functional changes within the organism, one or all four may reflect alterations in the fluid matrix and tissues of the body. In the synthesis of these important components of blood within the body there are certain determining, reacting, and participating factors to which all are subject; in addition, within the blood stream all are exposed to the same environmental influences. In hematopoiesis, granulocytes, erythrocytes and blood platelets originate in the myelogenous system (bone marrow); the lymphocytes in the lymphatic system (spleen, glands, dispersed lymphatic tissue); and large mononuclears and transitional forms or monocytes probably develop from the reticuloendothelium of spleen and liver, bone marrow and connective tissue.[290] A complete blood study is invaluable in differential diagnosis of disease, but it is increasingly evident that the normal biological state, influenced by various external and internal physiological factors, may encroach upon the borders of the pathological. The borderline becomes more obscure, then, necessitating more precise investigations of the predominating influences which may cause a physiological change in the blood, such as nutritional anemia (see p. 229).

In an investigation of the relationship of some of the measurements of the blood constituents we have found that statistical evidence from correlation tables for erythrocyte-hemoglobin, hemoglobin-leucocyte and erythrocyte-leucocyte relationships have established mathematically the precept that quantitatively hemoglobin content, red blood cell counts, and white blood cell counts are independent of one another within certain limits.[119] Although a rise in number of erythrocytes is usually accompanied by a slight rise in the quantity of hemoglobin, a knowledge of the red cell count is of little value in estimating the actual amount of hemoglobin. Similarly when the hemoglobin value is known, it is not possible to predict what the red cell count will be, nor will a white blood cell count be of any value in gauging the number of red blood cells, or vice versa.

Racial Differences

Racial differences expressed in hemoglobin values have been detected in the blood of negro and white infants[245] and adults.[66] In a study of 3469 blood samples taken from 364 healthy infants during their first year of life, 2563 observations were made on 272 white babies and 906 observations were made on 92 negro babies. The average values of hemoglobin for the white infants were from 0.5 to 1.0 gm. per 100 ml. of blood, higher after the fourth month than those for negro infants during the remainder of the first year of life. Figure 10 illustrates the hemoglobin curves and the racial differences observed. The hemoglobin measurements for the negro infants were characterized by significantly greater variation than those for the white infants during the period from the fourth to the twelfth month, during which the average for the negro babies was significantly lower. Confirmation of lower hemoglobin levels in the negro race has been

FIGURE 9. Representative subjects of infant growth studies.

made more recently by Dill and associates on white and negro men.[66] Despite the higher hemoglobin values for the white infants, we failed through detailed statistical study to establish any significant racial differences between the red blood cell counts of the white infants and those of the negro infants. A spontaneous variation in the red blood cell count and hemoglobin has been observed and the two values do not necessarily parallel each other.[314] There is a marked postnatal adjustment of the red blood cell count and hemoglobin in individual children.[116,244,372]

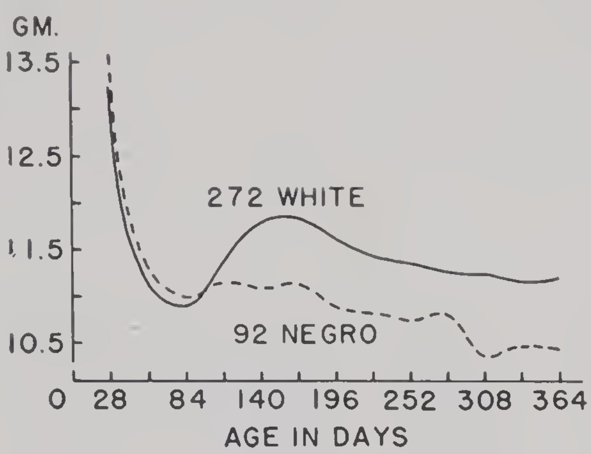

FIGURE 10. Hemoglobin differences in healthy white and negro infants.—*Reproduced by courtesy of the* American Journal of Diseases of Children.

Leucocyte Counts

The number of white blood cells may be taken not only as an index of physiological status of the tissues from which they are derived, but of structural and functional changes or abnormal disturbances within the organism. In interpreting the leucocyte count consideration should be given to the extent of individual variations, definite changes that take place as growth proceeds,

TABLE 5

WHITE BLOOD CELLS OF WHITE INFANTS*

Age	FEMALES			MALES		
	Individual	Determinations	Average count	Individuals	Determinations	Average count
months	number	number	per c.mm.	number	number	per c.mm.
1	20	77	9,700	42	108	10,100
2	35	98	9,700	32	102	9,900
3	16	58	9,100	32	64	9,100
4	23	36	9,800	36	54	9,800
5	32	43	10,600	38	51	10,600
6	31	44	10,700	46	61	10,300
7	31	41	10,500	45	58	10,400
8	23	44	10,900	37	57	10,600
9	26	44	11,300	39	63	10,200
10	22	47	10,700	27	49	10,200
11	10	30	9,600	24	48	10,700
12	17	43	10,400	18	56	11,200

* All infants were resting quietly when blood samples were taken.

the effect of specific environmental conditions, and pathological and physiological conditions upon the blood picture.*

In a study by this Laboratory 605 counts were made on 286 baby girls and 771 counts on 416 baby boys. Because there is still some question as to whether there may be a diurnal rhythm in the fluctuation of the leucocyte count in infants,[99,369] all determinations were made in our series between 10:30 A.M. and 12:00 M. (Table 5). This precaution was observed because individual

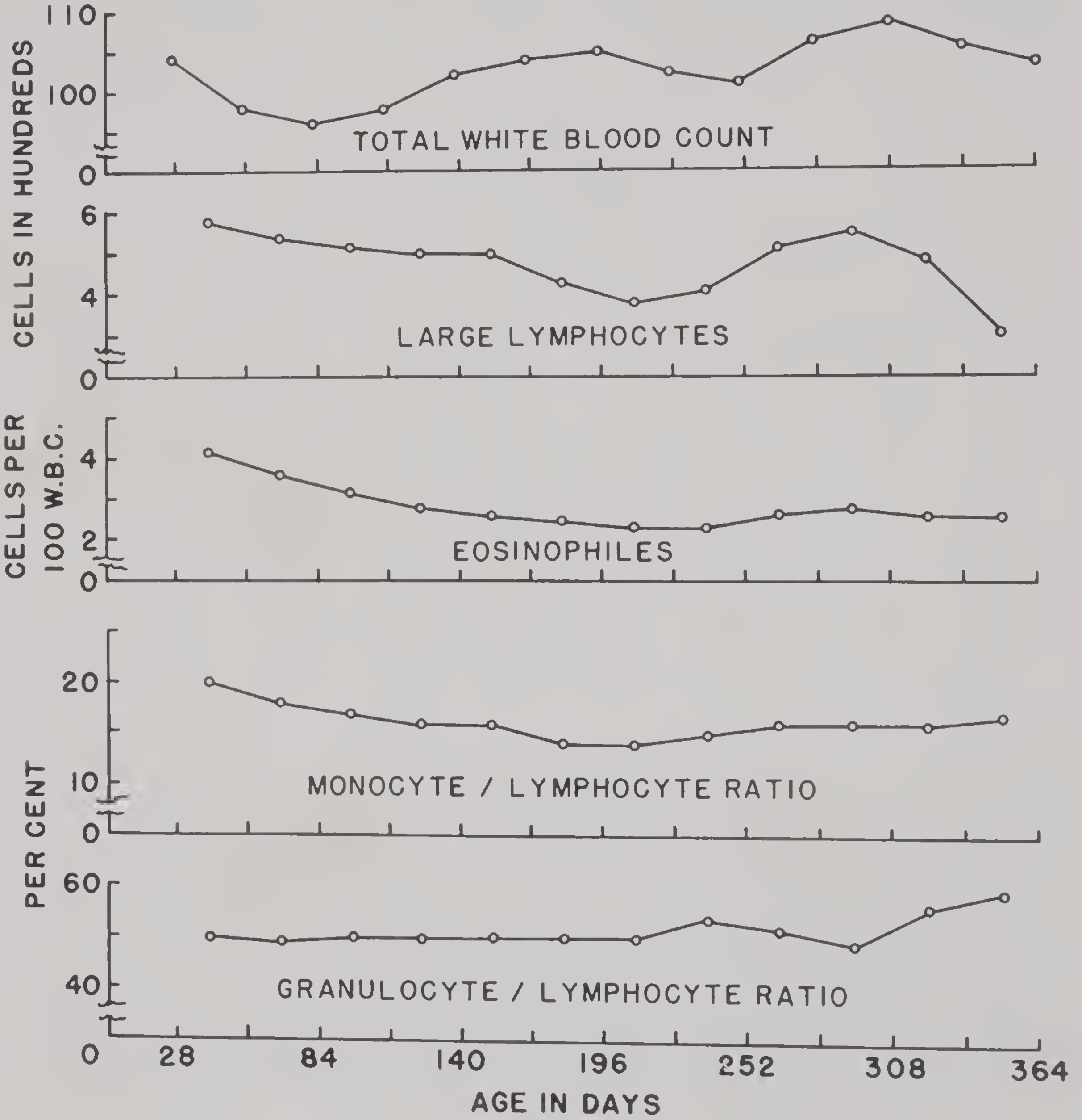

FIGURE 11. Total white blood cells and lymphocytes in blood of healthy infants; the percentage of eosinophiles and the ratios between the percentages of monocytes and lymphocytes and of granulocytes and lymphocytes.

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* A study of the white and differential blood counts during infancy and childhood as a possible criterion of nutrition. M. Lange, F. Kenyon, M. W. Poole, B. M. Hamil, and Icie G. Macy. Presented before the Ninety-Second Meeting of the American Chemical Society, Sept. 7–11, Pittsburgh, 1936. (Unpublished.)

variations in the leucocyte count of infants are so much greater than with other age groups, owing to the physiologic instability of the infant, that they might obscure any rhythm that might occur. The leucocyte count is one of the many illustrations of the instability of the structure and function of the young, incomplete individual.

Leucocyte Partition

The differential leucocyte count contributes special information on the physiological processes that are taking place in the body. The polymorphonuclear cells may reflect the status of the myelogenous system, the monocytes reflect the reticulo-endothelium and the lymphocytes that of the lymphatic system. Washburn[370,371] has investigated in a very thorough manner the differential white blood cell counts in infants from the second to the twenty-sixth week of life. He found fluctuations in all the counts of white blood cells during the hours of the day and night but no tendency to orderly rhythm; they vary from one day to another and show no correlation with age, activity, feedings or the routine events of the day. The curve of the lymphocyte counts is the only one which seemed to show a persistent tendency to parallel that of the total leucocyte counts. It has recently been shown[139] that at the onset of the fatigue phase after exercise there is a significant lymphocytosis suggestive of an

TABLE 6

DIFFERENTIAL LEUCOCYTE COUNTS FOR WHITE INFANTS

Values in per cent

Age in months	Number of Individuals		Number of Determinations		Polymorphonuclears		Large Lymphocytes	
	Female	Male	Female	Male	Female	Male	Female	Male
1	76	106	76	110	26.7	24.6	6.2	6.3
2	97	120	107	137	24.7	24.2	6.2	5.8
3	84	96	96	112	25.7	25.7	5.1	5.8
4	70	88	87	103	26.5	27.0	4.9	4.7
5	64	83	74	98	27.2	26.5	5.2	4.5
6	65	84	74	91	27.4	26.8	4.2	4.4
7	60	70	65	77	29.6	26.6	3.3	4.8
8	50	70	51	73	27.8	27.7	4.2	4.5
9	43	73	46	77	28.0	28.4	3.4	4.3
10	43	62	45	65	28.7	29.0	4.6	4.5
11	39	51	39	52	32.1	30.5	4.5	2.6
12	37	62	39	62	31.1	30.8	3.3	4.0

"irritation phenomenon" such as is frequently found in infections. It is important then that all counts be done under highly standardized conditions and under circumstances that will be representative of a quiet, calm, physical and mental state.[239]

Table 6, covering 1856 differential leucocyte counts on 1693 healthy infants suggests significant trends as the process of maturation proceeds during the first year of life. There is a change in the configuration of the eosinophiles, large lymphocytes, and the monocyte-lymphocyte ratio (Figure 11). The large lymphocytes have been plotted, using actual numbers, whereas the percentages were used in the other two cases. They never return to the original high level observed during the first six weeks of life. There is a gradual decrease in the eosinophiles, which arise in the bone marrow and spleen, and in the large lymphocytes, derived from the lymphatic system. The monocyte-lymphocyte ratio also diminishes; therefore monocytes are also decreasing. From a practical point of view it is significant to note that the granulocyte-lymphocyte ratio for an infant shows a definite rise at eight months of age, indicating an increase in granulocytes with a corresponding decrease in lymphocytes. A similar study has recently been reported by Kato.[172]

TABLE 6

DIFFERENTIAL LEUCOCYTE COUNTS FOR WHITE INFANTS

Values in per cent

Small Lymphocytes		Total Lymphocytes		Monocytes		Eosinophiles		Basophiles	
Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
49.9	52.0	56.1	58.3	13.1	12.2	3.8	4.6	0.3	0.3
55.8	54.8	62.0	60.6	10.3	11.0	2.6	3.8	0.4	0.4
55.6	55.2	60.7	61.0	10.2	9.8	3.0	3.3	0.4	0.2
56.8	56.2	61.7	60.9	9.0	8.8	2.5	3.0	0.3	0.3
55.8	57.0	61.0	61.5	9.0	9.1	2.5	2.6	0.3	0.3
58.1	57.5	62.3	61.9	8.0	8.7	2.0	2.4	0.3	0.2
57.5	57.8	60.8	62.6	7.5	8.3	1.8	2.3	0.3	0.2
57.1	56.4	61.3	60.9	8.7	8.6	1.9	2.5	0.3	0.3
56.8	55.2	60.2	59.5	9.0	8.3	2.5	3.4	0.3	0.4
55.1	54.0	59.7	58.5	9.2	9.2	2.2	3.0	0.2	0.3
52.3	54.3	56.8	56.9	8.4	9.2	2.5	3.1	0.2	0.3
54.9	51.9	58.2	55.9	7.9	9.5	2.5	3.3	0.3	0.5

Hemoglobin and Erythrocyte Counts

Table 7 records the average values of 1373 hemoglobin determinations and red blood cell counts on 1266 white infant girls and the average values of 1832 hemoglobin studies in 1681 infant boys during the first 12 months of life. Only healthy babies were included who were known to be consuming diets adequate in all nutritive essentials and were under special medical and nursing supervision.

TABLE 7

HEMOGLOBIN AND RED BLOOD CELL CONTENTS OF BLOOD OF WHITE INFANTS

Age months*	FEMALES				MALES			
	Individuals	Determinations	Average hemoglobin	Red blood cells	Individuals	Determinations	Average hemoglobin	Red blood cells
	number	number	gm./100 ml.	millions	number	number	gm./100 ml.	millions
1	77	79	13.1	4.34	106	113	13.1	4.30
2	126	143	11.3	3.74	163	188	10.8	3.69
3	146	161	11.0	3.94	170	194	10.9	3.90
4	145	172	11.5	4.29	172	193	11.6	4.30
5	134	150	11.8	4.50	169	193	11.7	4.66
6	124	135	11.7	4.60	164	180	11.7	4.75
7	107	111	11.5	4.67	141	152	11.4	4.87
8	96	97	11.4	4.71	131	136	11.2	4.81
9	88	92	11.4	4.78	134	140	11.2	4.84
10	86	91	11.3	4.68	124	132	11.1	4.90
11	76	80	11.2	4.80	104	108	11.1	4.92
12	61	62	11.3	4.65	103	103	11.0	4.86

* Age of an infant was determined by dividing the 365 days in a year into 12 months and converting ages in days to ages in months with this factor. When an infant came in twice for the same age interval the two values for red blood cell counts and hemoglobin values were averaged and the average value used instead of both values, to prevent weighting (i.e., if a child came in twice with only a short time interval between visits he might be three months old the first date and still be three months old the next time he came in, so these two values were averaged).

The number of individuals represents the different cases included. The number of determinations indicates the total number of determinations (this number will be larger because frequently the same child has two counts at the same age level).

Metabolic Balance Procedure

The metabolic balance is derived by calculating the difference between intake and output of a food constituent by the body. Intake is measured by analysis of representative samples of the diet and output is determined by analysis of fecal and urinary excretion. While the mineral balance is the only method by which the storage of minerals by living subjects may be investigated, difficulties in interpretation arise from the fact that it is usually impossible to measure directly the loss of water and chemical substances through the skin and there is no quantitative chemical method for partitioning the fecal content into the amount representing unassimilated food residue, that which is of bacterial or glandular origin, or is attributable to sloughing of the alimentary tract or the amount which results from direct excretion of products of metabolism (Figure 12) into the intestine.

The body does not always adjust itself readily and completely to changes in dietary, environment and routine. Also, there are physiologic lags inherent in the metabolic balance method of investigation, especially in the intestinal phase. With children, the extremely variable influence of growth is superimposed upon the other factors in physiological adjustment. The metabolic balance is a costly experimental procedure, expensive in both time and money and if the method is not applied under adequately controlled conditions, or if any part of the procedure is not carried out with exacting technique, the results are subject to question. Though the requirements of this type of investigation are extremely arduous, correct and thorough evaluation of the results is even more difficult, particularly with data obtained upon normal subjects. The experimental subject with some abnormality of body structure or physiologic functioning may provide metabolic balance data which clearly are abnormal; these data may deviate widely from data obtained simultaneously upon a normal subject used as a control; however, with-

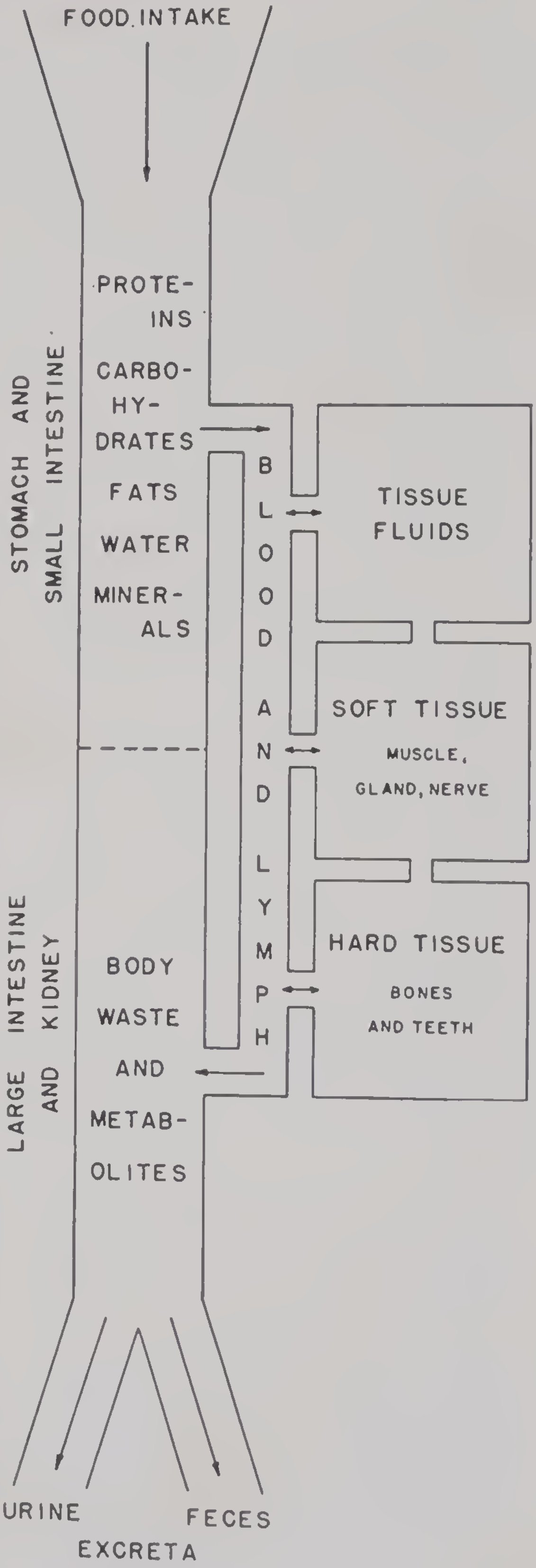

Figure 12. Schematic drawing of a metabolic balance.

out sufficient information upon normal individuals' range of metabolic response the significance of the results cannot be estimated.

In 1935, in a report of metabolic studies upon a child with osteopsathyrosis and another child with an ununited fracture[53] we were compelled to start our summary with the statement: "There are some striking variations from the normal in the retention of certain elements in both children, the significance of which cannot at present be definitely appraised." The dearth of metabolic balance data on normal children was the major contributing factor to the inability to appraise the experimental results obtained with the morbid subjects. The purpose of the intensive investigations of normal children, which have been carried out in this Laboratory for ten years, has been to obtain sufficient knowledge of the normal child to enable comparative evaluation of the metabolism of the ill child.

Length of Metabolic Balance Periods: In conducting a metabolic balance investigation, physical limitations of staff and equipment preclude analysis of samples representing each single day's food intake and fecal and urinary output. In addition, separation of the feces representing each single day's food intake would present an enormous problem and would involve unavoidable variations attributable to the separation. Urine collections adequately represent metabolism during the interval immediately preceding collection but food particles fed to human beings may not be passed in the feces for several days. In early studies of relationships between food intake and excretion these difficulties were recognized and investigators tried various methods of distinguishing the feces representing a given period of food intake.

Voit[206] fed bones to a dog eighteen hours before starting an experiment and again at the conclusion of the investigation. The bones formed a white excrement in the feces, indicating the first and last portions of feces from the experimental diet. Rubner[206] gave two liters of milk to a man eighteen hours before the start of an experimental feeding period. The milk produced a distinct dividing line in the feces. Cremer[206] used freshly precipitated silicic acid (10 to 25 gm. mixed with 40 to 100 gm. fat), which gives excellent results, as it avoids the protein nitrogen in the bones, and is of great advantage if calcium or other ash constituents of the feces are to be determined. Carmine and lampblack are most frequently used to separate the feces and of these we have preferred to use carmine, for small amounts of the red coloring are distinguishable, even in very black fecal material.

Feces resulting from the food intake of several days may be composited, reducing the errors which would be inherent in daily separation and any physiological effect of the substance used as a marker (Figure 13). A study of the effect of carmine upon the gastrointestinal response of normal children has provided some evidence that carmine influences the response of the gastrointestinal tract and the wet weight of the feces (p. 104). Because of the evidence that carmine may have some effect upon digestion and elimination, it has been our conviction that it should not be given too frequently.

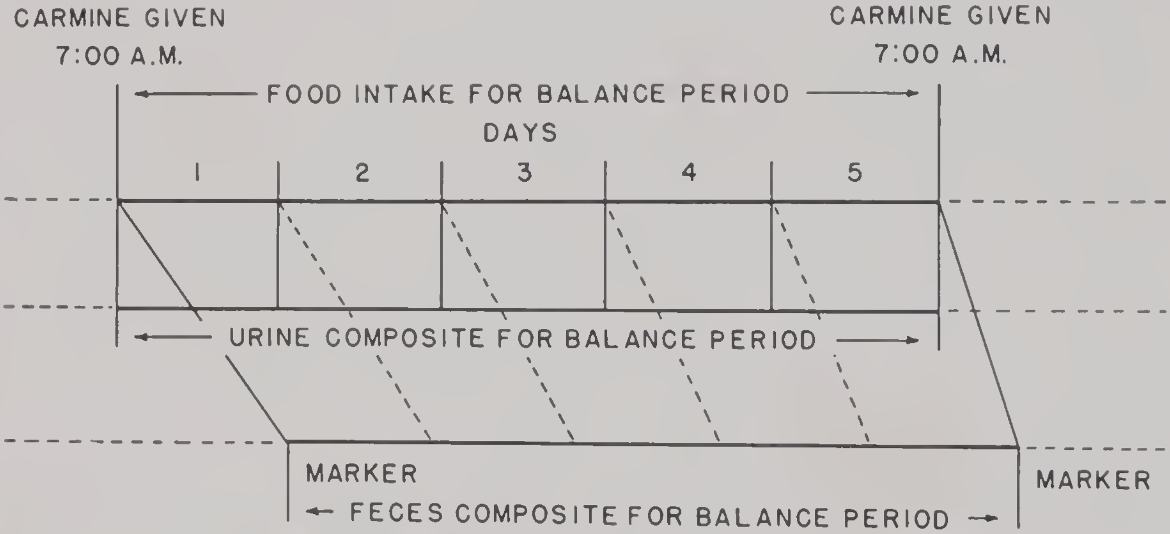
CARMINE GIVEN
7:00 A.M.
CARMINE GIVEN
7:00 A.M.
FOOD INTAKE FOR BALANCE PERIOD
DAYS
1
2
3
4
5
URINE COMPOSITE FOR BALANCE PERIOD
MARKER
MARKER
FECES COMPOSITE FOR BALANCE PERIOD

FIGURE 13. Schematic drawing of collections for a metabolic balance period.

Duration of Metabolic Balance Studies: The length of metabolic balance investigation required to obtain results of physiologic significance is not known. With morbid subjects, results considered significant are frequently obtained from one three-day metabolic balance period. The length of investigation needed is greatly influenced by the type of subjects to be studied (infant, child or adult; normal or abnormal) and the extent of their preparation for study. Individual variations in physiological activities have been indicated[155,159,218,220] and we have emphasized adequate sampling periods in metabolic studies.[67] Length of both the balance period and the total observation period are of prime importance in metabolic balance studies. Balances including only a few days register fluctuations in metabolic processes that longer periods, although valuable in showing general tendency, tend to conceal. A longer observation time, divided into short balance intervals of a few days each, has merits of both the short and the long balance period. Since the desirable length of time is not known for obtaining factual information on growth we have obtained successive metabolic balances for weeks at a time at different intervals of the growth process. Continuous observations possess accuracy and significance unquestionably greater than would characterize any short period of observation. We have used five-day metabolic balance periods as a compromise between giving markers too frequently and making chemical determinations too far apart.

In 1932, a study of six children for 25 to 65 successive days, while they received identical and unchanging diets, showed individual trends of calcium storage which were neither related to age nor to body size. We stated:[159] "Obviously it is necessary to learn throughout a pre-experimental period of several weeks how wide these customary physiological fluctuations may be for a specific child as well as the individual rates of storage under highly standardized and desirable conditions before the effect of any regimen upon the metabolic balance can be satisfactorily understood. Without the inclusion of such controlled data one may be easily misled into false fields of interpretation by the use of this method for determining the subsequent effect of certain foods or other factors on metabolism."

Subsequently, in 1933, a continuous metabolic study on children was published by Porter-Levin[270] in which she noted wide variations in the retentions of calcium and phosphorus from period to period and remarked: "An inspection, as well as statistical treatment of the two longer series of retentions, indicates that it required from five to seven successive balances, or from 15 to 21 consecutive days, to cover the entire range of variation in these retentions." From our own experience a period of 21 days will not always suffice to reveal differences among individuals, nor in any one individual at all times.

On another occasion we stated that "more intimate knowledge of the functioning of metabolic processes under differing physiological and environmental conditions offers opportunities for further investigations. The selective action of the body in digestion, absorption, utilization and excretion of the ingested foods has not as yet been elucidated. Extreme variations in the balances of consecutive periods must of necessity require a marked compensatory action of the body to supply sources of supplement for losses and means of storage for excesses. Each additional experiment contributes to a more complete knowledge of the factors that operate to influence metabolic behavior and to a more intelligent interpretation of these factors."[67]

Standardization of Subjects: In all metabolic studies, standardization of the experimental subjects is dependent, initially, upon selection of comparable individuals. With subjects who are to be studied owing to disease or abnormal function, very little can be accomplished in standardization beyond the selection of procedures. In such studies the interpretation of results depends upon comparison of the data with those obtained simultaneously from a "control" group of individuals, or with values available in the literature. With normal individuals, control may be established by studying sub-groups under different conditions and contrasting the results, or the individuals may act as their own controls and interpretation be made from the variations among individuals and of the same individuals from period to period.

The wide natural variations in physiologic response among normal individuals have impressed us with the impossibility of close standardization of two groups of normal children. If large numbers of subjects can be used, the natural variations of the individuals influence only slightly, and perhaps not at all, the interpretation of the results, but when only a few subjects are studied the question of close standardization between two small groups is eliminated by using the same subjects for both control and experimental procedure. This has certain disadvantages also, for the child is changing in size as growth proceeds[40] and changes occurring as the result of the length or sequence of the pre-experimental (control) and experimental periods of observation must not be attributed to the factors under investigation.

Subjects of metabolic balance investigation can be standardized to some extent, preliminary to actual study; the individuals can be established in the environment in which the observations are to be carried out, the routine of the study can be initiated, and diets given which will insure ample quantities of all food constituents. Even after all these efforts to obtain standardization, further standardization may occur during the course of metabolic studies over extended intervals.

In the course of a metabolic balance observation extending over eight months when ten children received diets composed with the same foods, in daily quantities considered adequate and containing an average of 0.9 gm. calcium per day, the interesting phenomenon of apparent standardization appeared among all the children (ages four to eight years). As the study proceeded the children, regardless of age or size, showed less variation from each other and they all became more alike in the amount of calcium they were able to retain daily (Figure 14). Since the study was carried for only eight months it is not known whether the subjects would have become even more nearly alike in their selective retention of food constituents, and therefore in their daily retentions of this element, irrespective of body size, nor is it possible to determine whether season was an influencing factor. There was not only stabilization of the daily retentions of calcium but also there was a gradual increase in average quantity retained by the group as the months passed.

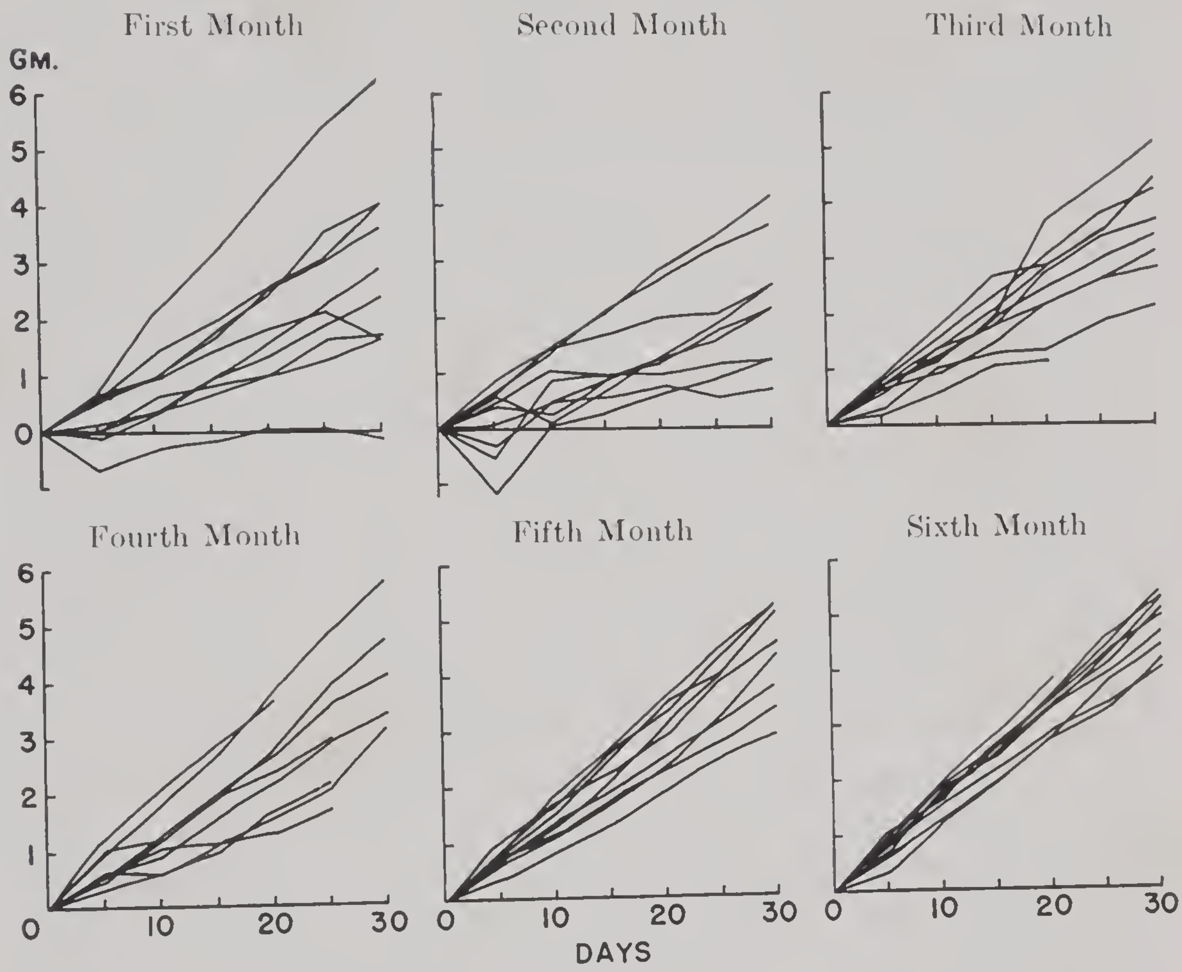

Figure 14. Cumulated calcium retentions for consecutive months. Each curve represents the retention of one child for one month.

Dietary Considerations: Some investigators feed each experimental subject an amount of each food element proportional to his body weight,[129] thus hoping to give the same number of calories and grams of each mineral constituent per kilogram of weight, that is, all the children do not have the same total amount of milk, egg or other foods, but they receive practically the same quantity of individual food components per unit of body weight. Such a procedure is based upon the assumption that the chemical composition of a kilogram of body weight is the same for all individuals and for the same individual at different times.[120] Since it is known that body weight is a fluctuating entity and may not represent a constant body composition, because of the labile fat and shifting of water in and out of the tissues, this method of feeding has certain disadvantages. In addition, fresh foods vary so much in their chemical composition that it is difficult to predict how this variable factor may affect the number of calories and grams of each mineral constituent when fed on the basis of a kilogram of body weight.

That weight alone is not concerned with the variations in nitrogen retention of pre-school children has been demonstrated. High retentions were found among children who were above the average in weight as well as those below the average. Indeed, Daniels and her associates[61] have emphasized the inherent differences in requirements of children destined to be tall or short, and fluctuating needs from time to time.

My associates and I have had the conviction that since basing food intake upon the per kilogram of body weight assumed that there is a direct relationship between body weight and the quantity of all food constituents utilized, it is more exact to give each subject a definite quantity of food each day, sufficient to meet fluctuating physiological needs, and observe how it is metabolized.[220]

Variations in the Sampling of Foods: One of the most exacting tasks in metabolic balance studies is obtaining accurate chemical analysis of a diet representative of the one consumed by the experimental subject. The precision and value of metabolic data depend to a large degree upon this important phase of the work. Numerous methods are in vogue for estimating the chemical composition of the dietaries, the choice of method usually being governed by the availability of laboratory equipment and facilities and the number of trained workers engaged in the labor. An investigator may estimate the amount of each of the elements in the diet from the raw weights of the individual foods, using average values found in tables in the literature; analyze chemically the individual foods used in the diet and calculate therefrom the composition of the diet; analyze a mixed diet analogous to the one consumed by the experimental subject; or, the investigator may weigh aliquot portions of each item of food at the time the diet is prepared and then analyze a pooled sample representative of the diet for a day or for a metabolic balance period.[5] Because these methods provide data of such varying degrees of accuracy, it is difficult to compare the metabolic balance results obtained in one laboratory with those of another.

Sampling for Metabolic Balance Determination: It would be ideal if all metabolic specimens could be completely analyzed in the fresh state as they accumulate each day, but the analytical task is too great and certain methods are too laborious to permit its accomplishment. Furthermore, fecal material for analysis must be accumulated over the entire metabolic balance period, whether it be three, five, seven, or ten days. Some samples must be analyzed in the wet state, others in the dry. The accuracy with which the feces are separated, period by period, the care with which their moisture content is preserved, and oxidation and bacterial action prevented until the samples are ready for analytical treatment, all contribute to the accuracy of the final metabolic balance results. Certain elements such as nitrogen, sulfur and chlorine are dissipated in the open-air, heat-drying process, some constituents such as fatty acids may be decomposed, and still others may be changed to such an extent that accurate analyses become impossible.

Since metabolic balance values contain the combined errors from collecting and sampling the foods and excreta and the methods employed in analysis, it is essential to exert every effort to simplify, standardize and apply the most accurate techniques, thus minimizing all possible sources of error, if the balances are to have scientific merit. It long has been known that variations in retention occur and it is essential that some measure be available as to what part of the retention figure must be attributed to variations inherent in the diet, before attempting to correlate changes in retention with other factors. Sporadic observations of too short duration, poorly organized procedures of collecting and handling metabolic specimens and unsatisfactory methods of chemical analyses, either singly or in combination, may not only forfeit the most sanguine explanation of Nature's behavior but may actually lead one astray into false fields of interpretation.

With the accumulation of data on the chemical composition of foods, carefully sampled and analyzed by highly refined and more accurate methods, it has become increasingly apparent in recent years that common foods vary widely in composition, particularly in their mineral constituents.[393] Several papers[5,129,217,368] describing the technique of conducting metabolic experiments have emphasized the fact that a given diet must be analyzed under the precise conditions of the experiment at hand, if the values for its mineral or nitrogen contents are to be used with any assurance of accuracy. A survey of the literature on the mineral content of common foods[393] has pointed out that while the calcium, phosphorus, and sulfur contents of some foods vary by only 25 per cent or less, the amounts of the same elements in other foods vary as much as 200 per cent. This implies no criticism of the validity of the analyses, but merely indicates that there are differences in individual composition, even among specimens of the same variety. With fruits and vegetables, these discrepancies have been attributed to ripeness, species, type of soil, mode of cultivation, amount of water available, geographic origin and marketing conditions. Attempts to correlate the variations in foods have been made[18,51,63,117] but the information is far from complete.

In addition to the inherent variability of foods as purchased, large losses of organic and inorganic constituents are found in varying degrees according to the method of cooking,[210,266] therefore, it is necessary to standardize the preparation of food from day to day in such a way as to preserve nutritional value of the diet and to serve the food in the individual dishes in which it is cooked, in order to assure consumption of food, equivalent to the dietary analyzed. Even the simple process of squeezing the juice from oranges may considerably alter the course of metabolism, for up to 50 per cent of the vitamin C content may be lost by the open air use of iron utensils.

Since there are unavoidable variations among identically prepared samples of raw foods, it is possible that even though great care is used in the preparation of duplicate dietaries for chemical analyses and consumption by the subject, the sample dietary analyzed may be quite different in chemical composition from the dietary ingested by the subject. Obviously, such variations in food intake alter the composition of excreta. It would seem, then, that in metabolic balance studies the average composition of the food intake, as determined by analyzing duplicate dietaries at frequent intervals, would give representative values for food intake, and consequently provide the best data for use in calculating retention.

CHAPTER III

ORGANIZATION OF AN EXTENSIVE METABOLIC INVESTIGATION

CONTROL of an investigation carried on with the ill or abnormal subject is simplified by the restraint which his illness or abnormality places upon body activity and environment. An extensive and rigorous study of average healthy subjects must, in its conception, be predicated upon maintaining study conditions which approximate acceptedly normal physical environment, subject to the usual strains of mental and emotional influences, at the same time allowing for the accuracy of control necessary to obtain consequential data. In working with adults, selection of cooperative subjects and provision for adequate training and supervision are the essential conditions for proper investigation. In studies with infants the restrictions of the crib simplify environmental control procedures.

The most difficult studies of nutrition and chemical growth are those which involve normal children who are beyond the age of crib or playpen restriction, but not yet old enough for dependable cooperation in the absence of supervision. Control in such studies fundamentally is dependent upon procuring the cooperation of the boys and girls by stimulating their interest and pride in the work. On the following pages we present in detail the organization and maintenance procedures which were utilized in the controlled investigation of normal children between the ages of four and twelve years during periods of as long as eight consecutive months. With other subjects, either older or younger, many of these procedures may be simplified.

The Methodist Children's Village, only 12 miles from the Research Laboratory of the Children's Fund of Michigan, provided the requisite situation in which the biological processes of children might be studied (see views inside cover). Unlike many institutions, the Village does provide a home. The youngsters live in groups of nine to twelve, with a housemother experienced in handling children. Each of these groups makes up a separate family unit in one of the modern houses widely spaced upon the forty-acre grounds. The property is attractively landscaped and the tree-lined Rouge River cuts directly through its rolling acres. Business and administration are confined to the administration building at the entrance of the grounds and the rural location has required that the Village build and maintain a modern school.

Some of the children who live at the Village are orphans for whose care the organization has voluntarily assumed responsibility, others have been placed in care by their parents or guardians. Parents unable to provide a home for their children, owing to ill health, economic conditions, or separation utilize the facilities provided by the establishment, contributing financially to its support if they are able to do so. During an investigation the children who participated lived in one of the "cottages" with a housemother and three young women trained in metabolic routine. Hours were arranged so that each person had one entire day off each week.

Equipment for Metabolic Study with Children

Several weeks before an experimental regime was started the children were established in the cottage and the necessary additional equipment installed. An analytical balance for weighing foods and graduates for measuring water were kept upon a side table in the dining room. An electric refrigerator placed near the bathroom was used only for keeping the day's collection of excreta; a beam balance for determining body weight was set up in the basement; the instruments for making anthropometric measurements, the basal metabolism equipment, and a measuring board for obtaining recumbent lengths were placed in a separate room; and, the individual collection, kitchen and dining room utensils were placed in service. Wooden commodes were made, suitable to the size of the children, one with a shelf beneath the toilet seat for the jars in which feces were collected, and another, without a shelf, for collection of urine into jars, through glass funnels.

Ample supplies of glassine bags were necessary for individual one-day allotments of salt, and since each child ate and drank from individual containers marked with his name, the following equipment was necessary for each child: one enameled metal tray 12×29 inches; three, 200 ml. bakelite glasses; one, 300 ml. porcelain casserole; three, porcelain sauce dishes (about 100 ml.); two, white, graniteware, flat-bottomed pans (about 50 and 100 ml. capacities); one, shaker-top, glass sugar jar (200–300 ml.);

two, 500 ml. Florence flasks, equipped as water bottles; silverware; a small spatula; and several glass stirring rods.

Standardization of Children in Metabolic Studies

With the environment and routine of the studies established at least two weeks in advance of actual collections, the interval served to stabilize the children; their social adjustments to each other and to the technicians and housemothers were made, they were accustomed to the use of the collection equipment and regular elimination habits were stimulated. By the time the regular regime was instituted the children had overcome any tendency toward embarrassment and had been familiarized with all the procedures.

Diets composed of the foods to be used in the investigation were given during the preparatory period and the interval allowed the children time to become accustomed to the manner in which the foods were prepared and served, to drinking distilled water and to the group of foodstuffs which would be used. In addition, the children's quantitative requirements were determined during this time, so that they would be able to eat all the food given them and yet not have an unmet demand from hunger.

Supervision and Routine

The housemothers selected to supervise the children were capable, middle-aged women experienced in the ways of children and intelligent and resourceful in handling them. Mother "K," as the children called her, directed the daily routine of living—sleeping, dressing, bathing, eating, and studying—and preserved happiness and serenity among the children. Three young women, all trained in nutrition, constructed the menus, weighed and prepared all foods and fluids for consumption, assisted the housemother with the children during meals, supervised collection of the metabolic specimens, and assumed responsibility for accomplishing the scheduled procedure. Although the children were subjected to strict routine and supervision for long periods of time, every effort was made to preclude any psychological effect and to maintain a condition socially comparable to that which would have prevailed under conditions which were not experimental.

The children attended the private school maintained on the

Village grounds, where special arrangements could be made to maintain observation and collect all metabolic materials. The children valued the privileges which accompanied their participation in the experimental work and when questioned about their prerogative to leave the classroom temporarily would explain this with the statement that they were "speriment" children. During the week the children arose at seven o'clock each morning, washed, dressed, and brushed their teeth with just water. Before actual initiation of the investigation the children were trained for two to three weeks in regular bowel habits and throughout the studies constipation did not occur. After school, the children were allowed to play outdoors until dinner time, all days when the weather permitted, and following dinner were allowed to play outdoors until dark. During inclement weather the children played upon the roomy porch of the house, in the large basement, or in the living room. In the evening they entertained themselves with books and games and frequently enjoyed stories read to them before the fireplace by Mother "K" or one of her associates. The children had individual beds in light airy rooms and separate wardrobes and dressers for their clothing (Figure 15).

To maintain morale, efforts were constantly directed toward providing the children with privileges, "treats," and entertainment equivalent in value, in their opinion, to the sacrifices which they were asked to make for the study. To offset the dietary restrictions imposed by the routine, the nutritionists made pies, cakes and candy from the foods in the usual daily diet. Graham crackers were utilized for pie crust and cereals formed the base of cakes. A child's birthday was always an occasion for a party and upon holidays such as Halloween and Christmas the celebrations included appropriate dress, decorations and entertainment. At many times during the investigations one of the laboratory staff members or their friends entertained the children with pictures or talks.

While considerations of economy and consistency in the diet made it necessary to use the same common foods throughout each period of observation, in compiling the meals the ingenuity of the dietitians produced a variety of appetizing preparations, and identical menus were repeated not more frequently than once a month. The subjects received an ample amount of mixed food to meet their chemical requirements and satisfy their hun-

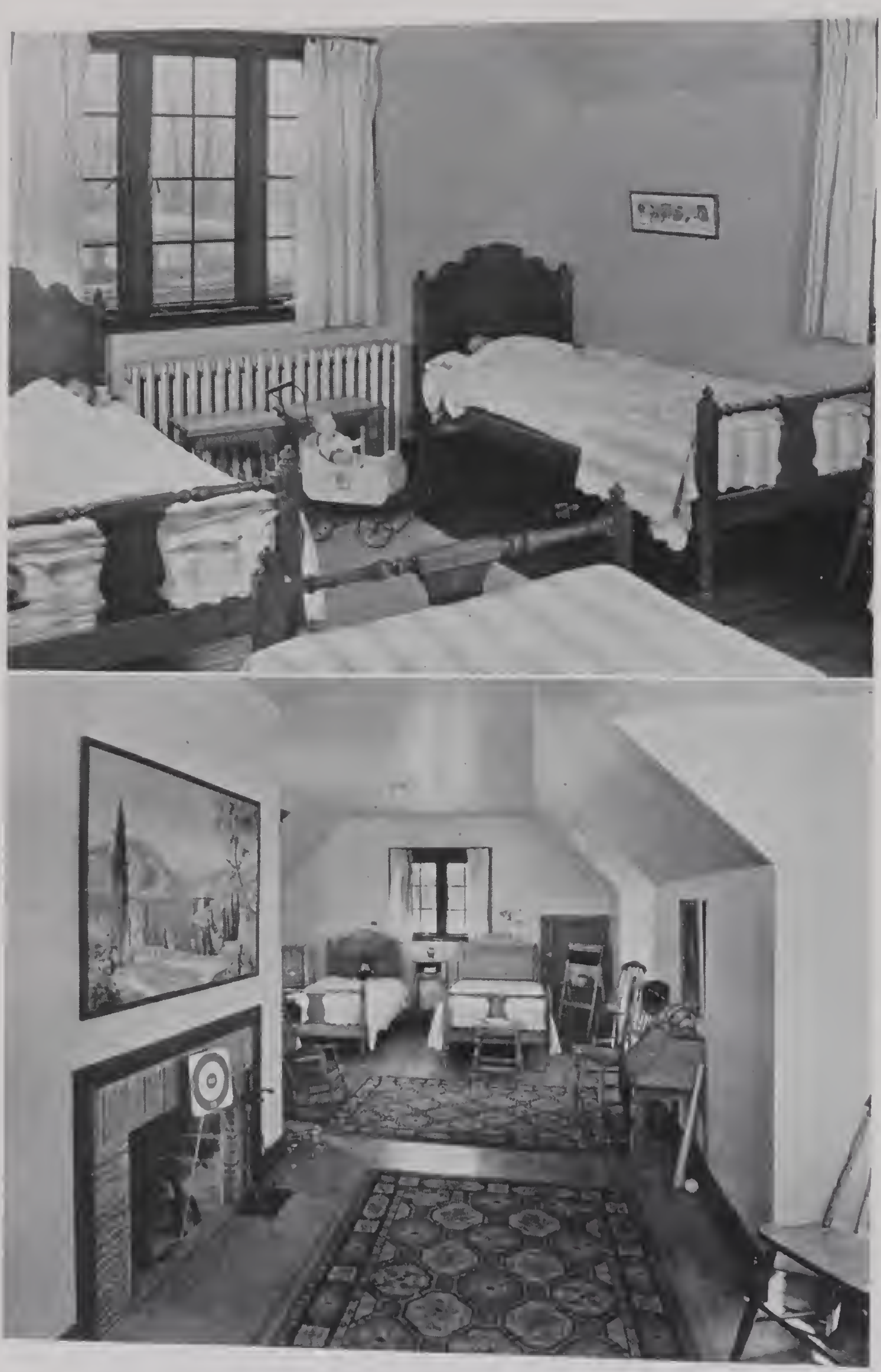

Figure 15. Sleeping quarters in cottage at Children's Village.

ger. Each child's tray was attractively arranged and the children, housemother and nutritionists ate together in the pleasant dining room of the cottage (Figure 16).

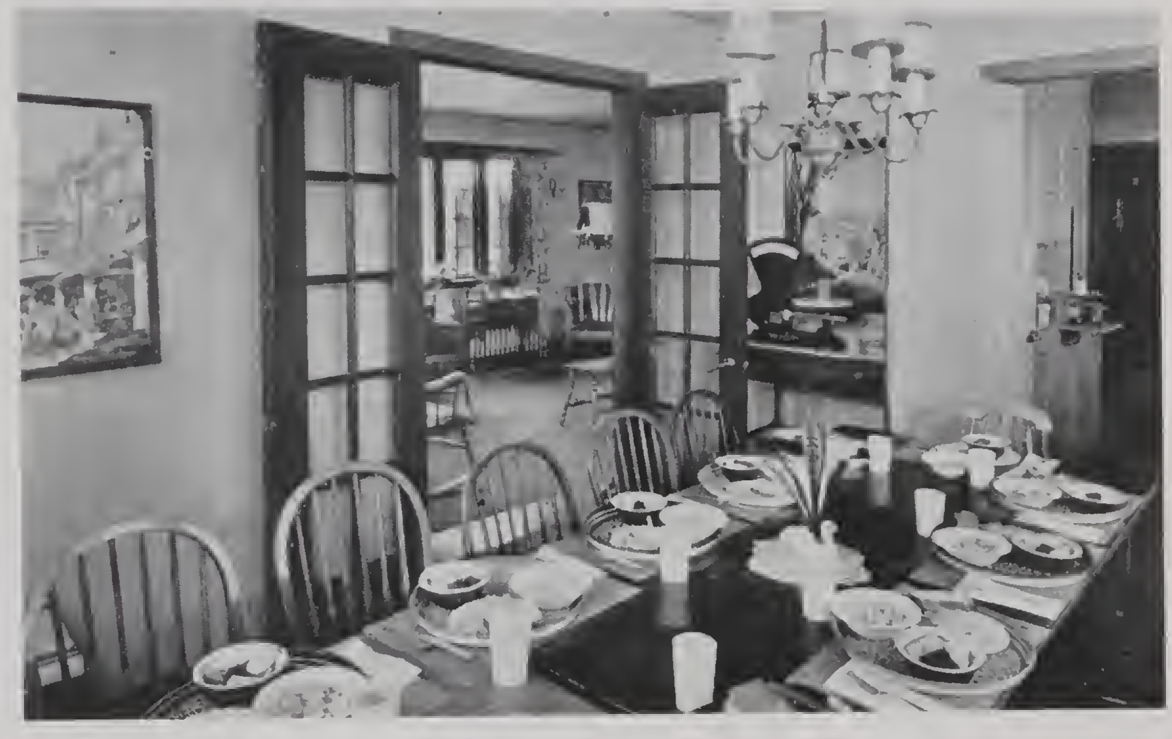

FIGURE 16. Dining room and food service in cottage used by the experimental subjects.

Food Sampling

In the metabolic studies, the foods subject to wide variation in composition were sampled daily. The first day of each balance period approximately one-half of the day's allotment of each variable food was placed in the refrigerator in a clean, covered glass jar. On each of the following days of the period an approximately equal amount of each variable food, was added to the proper jar. In a recent study, separate jars were required for apple, banana, cabbage, carrot, lettuce, and beef. At the end of each five-day balance period, each jar's content of an individual food was thoroughly mixed and one day's quota of that food accurately weighed and transferred to each of two, clean, covered glass jars sufficiently large to contain an entire composite.

If the food had been served raw on two days and cooked on three days, three-fifths of the portion was cooked as before serving. The other foods used were subject to far less variation in content, because of manufacturing and purchasing (see p. 77) control and the small amounts used in relation to the quantities purchased. After compositing the amounts of variable foods equivalent to one-day portions, daily allotments of each of the other foods in the diet except butter, milk, potato, salt, sugar and water were added, completing two identical composites,* each equivalent to one day's intake of the foods but representative of the food ingested each day of the balance period.

Butter, milk, and potato were not pooled with the other foods in the composite, principally because their bulk and composition would make preparation for analysis more difficult. In addition, these foods were among those given in varying amounts to the different children according to their age and size. Milk and potato were analyzed separately for each balance period. As an additional check upon the values determined upon the composite, all the individual foods were analyzed individually at the beginning and end of the study. The salt used was chemically pure sodium chloride. Distilled water was used for drinking and cooking. Salt, water and sugar were excluded from the sampling for analysis.

Collection of Excreta

Complete and understanding cooperation on the part of the subjects is necessary in collecting all excreta and in obtaining urine and feces in separate jars. Excrements were collected in glass receptacles, marked with the individual's initials and the date, covered with close-fitting glass covers and kept in a refrigerator. Individual stools may be collected in straight-sided glass jars, about five inches in diameter and seven inches high, with close-fitting glass covers (Figure 17). It is convenient to collect urine through an eight-inch glass funnel into a bottle labelled for the subject and transfer immediately to a two-quart Mason jar (containing one-half inch of toluol) in which that child's complete twenty-four-hour collection is being accumulated (Figure 17). The original bottle and the funnel are washed into the two-quart jar with distilled water from an individual wash bottle. Each day 150 ml. of distilled water are placed in each subject's wash bottle and the entire amount used in making the transfers for the day. The excreta for the preceding twenty-four hours was

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* One composite of the dietary provides sufficient amounts of material for all analyses but the additional composite facilitates the preparation of analytical material (see p. 237).

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removed from the refrigerator and transported to the laboratory every morning. In turn, a fresh supply of chemically cleaned, dry glassware was delivered daily to the cottage.

Observations and Determinations

The premise that subjects of human investigation must be, to a large extent, unknown quantities, regardless of the care with

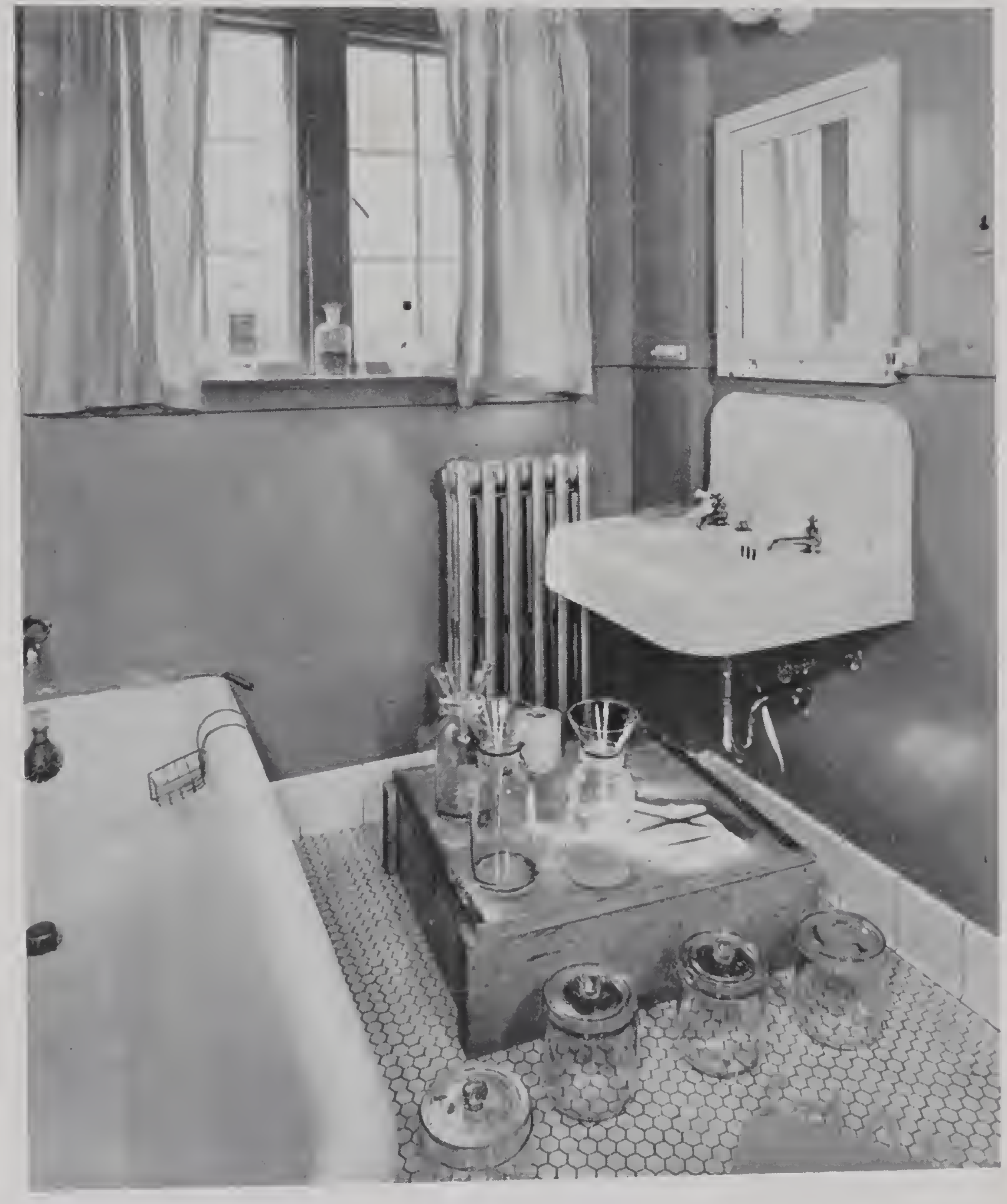

Figure 17. Equipment for collection of metabolic samples.

which the criteria for their selection has been chosen, is fundamental. The irrevocable involvement among growth, development and nutritional status prohibits, at present, delineation of methods for the determination of any one of these. Such methods as are applicable are dependent upon the familiarity and skill of the worker, the facilities at his disposal, the cooperation of the subjects and the conditions under which the samples are collected and chemical determinations are made. The accomplishment of many procedures is dependent upon their syncronization within the limits of time and resources.

The metabolic observations obtained with the children are shown in Table 8. Determinations were made of water, nitrogen, energy, fat, and 12 inorganic elements in food, urine and feces. The inorganic elements included the positive minerals, calcium, magnesium, potassium, and sodium; the negative minerals, chlorine, phosphorus and sulfur; and the heavy metals, copper, iron, manganese, nickel, and zinc. Polarographic methods were developed for determination of some of the heavy metals and some elements were determined upon the same samples by both polarographic and chemical methods.

It is essential in laying the plans for an extensive investigation of long duration to provide a staff of sufficient numbers and training to carry the work with equanimity. Each investigator should possess a moderate degree of tranquility and be specially qualified to carry a particular phase of the work with unquestionable rapport and credence. Several people should be not only familiar, but skilled, in any one determination or phase of the work, so that the program will not be disrupted in case of illness or unexpected absence of some one of the staff. The investigators responsible for managing children should have a real love for children and know some of the fundamental principles of child care and management. The best laid plans can easily go astray in the hands of the inexperienced, unqualified, easily excitable and over-taxed worker. The accuracy of the values obtained by group effort is only as satisfactory as the weakest link in the whole chain of events.

Selection of Normal Experimental Subjects

In gathering information upon nutrition and chemical growth in childhood three major investigations were carried out. In the first, 95 days, 11 subjects were observed (Table 9). Four years

TABLE 8
SUMMARY OF CLINICAL AND BIOCHEMICAL PROCEDURES

Observations

- Activity (pedometer)
- Emotional reaction
- Laxation rate
- Elimination time
- Dental condition
- Basal metabolism
- Respiration rate
- Blood pressure
- Body temperature
- Pulse rate
- Postural photographs
- Footprints
- Food sensitization
- Intelligence quotient

Roentgenological

- Fluoroscopic examination
- Gastrointestinal studies
  - Emptying time of tract
  - Gastric emptying
    - Influence of
      - fat
      - milk
      - meat
      - carbohydrate

- Full skeleton
- Teeth and jaws
- Chest
- Hand
- Elbow
- Shoulder
- Foot
- Knee
- Hip

Measurements

- Weight
- Recumbent body length
- Recumbent stem length
- Tibial length
- Biacromial width
- Intercristal width
- Intertrochanteric width
- Head circumference, length, width
- Chest circumference, length, width
- Upper arm circumference
- Abdomen circumference
- Subcutaneous fat

Chemical Determinations on Food, Urine and Feces

- Total solids
- Water
- Fat
- Nitrogen

Positive Minerals

- Calcium
- Magnesium
- Sodium
- Potassium

Negative Minerals

- Phosphorus
- Chlorine
- Sulfur

Heavy Metals

- Copper
- Iron
- Manganese
- Nickel
- Zinc

- Carbon
- Energy
- Carbohydrate
  - Total
  - Complex

Other Chemical Determinations

Feces

- Fat Distribution
  - Soaps
  - Neutral
  - Free fatty acids
  - Unsaponifiable

- Complex Carbohydrates
  - Lignin
  - Cellulose
  - Hemicellulose

Urine

- Sulfur Partition
  - Total
  - Neutral
  - Ethereal
  - Inorganic

- Volume
- Urinary *p*H
- Specific gravity
- Total acidity
- Organic acids
- Total solids
- Energy
- Carbon

- Nitrogen Partition
  - Total
  - Ammonia
  - Urea
  - Uric acid
  - Creatine
  - Creatinine

Blood Determinations

Red Cells

- Sodium
- Chloride
- Potassium
- Cell measurements
  - Resistance
  - Volume
  - Diameter
  - Weight
  - Thickness
- Neutral fat
- Free cholesterol
- Cholesterol esters
- Cerebrosides
- Phospholipids
  - Cephalin
  - Lecithin
  - Sphingomyelin

Whole Blood

- Hemoglobin
- Red cell count
- White cell count
  - Total
  - Differential
- Platelet count
- Specific gravity
- Total solids
- Total nitrogen
- Calcium
- Phosphorus

Plasma

- Specific gravity
- Total nitrogen
- Phosphorus
- Sodium
- Chloride
- Potassium
- Neutral fat
- Free cholesterol
- Cholesterol esters
- Cerebrosides
- Phospholipids
  - Cephalin
  - Lecithin
  - Sphingomyelin

later, 11 subjects were investigated during a period of 225 consecutive days, and after another four years, seven of the same children again cooperated, and determinations were made over an interval of 55 days (Table 10). The initial investigation produced extremely valuable data and, in addition, provided the experience and training which made it possible to carry on a more intensive study for almost eight consecutive months. Additional procedures were used in the selection of the experimental subjects and the scope of investigation was broadened. Many of the determinations performed as selective measures were repeated after the metabolic balance collections had terminated, and clinical examinations, including height and weight measurements, were made at intervals during the four years between the second and third studies. In the latter study the techniques and methods employed attained their greatest refinement and the results their greatest accuracy.

TABLE 9

GENERAL CHARACTERISTICS OF EXPERIMENTAL SUBJECTS IN STUDY I

Subject	Sex	Chronological age	ANATOMIC AGE				Stanford-Binet I. Q.	Recumbent length	Weight
			Based on hand			Todd§			
			Todd*	Flory†	Kelly‡				
number		mo.	mo.	mo.	mo.	mo.		cm.	kg.
1	M	47	—	—	—	—	—	108.6	18.60
2	M	58	69	41	<60	66	84	108.0	19.50
3	M	59	—	—	—	—	—	111.8	20.20
4	M	59	51	29	<60	51	—	99.1	17.99
5	F	66	59	62	—	58	96	106.7	17.57
6	M	70	72	65	78	72	115	111.8	19.65
7	F	78	—	—	—	—	—	109.4	21.06
8	M	80	79	67	90	80	137	114.8	22.30
9	F	99	93	78	—	90	110	125.3	24.20
10	F	107	99	84	—	101	—	128.3	25.27
11	M	123	152	109	138	153	—	134.0	33.67

* Averages of ratings by Todd and Francis according to Todd method of assessing differential skeletal maturation.[350]

† Determined by method of Flory.[100]

‡ Determined by method (for boys only) of Kelly.[175]

§ Averages of ratings by Todd and Francis for six age assessments based on roentgenograms of the foot, hand, elbow, knee, shoulder and hip.

In selecting the experimental subjects of the investigations of nutrition and chemical growth every available procedure was employed to determine that the designation "normal" could properly be applied to the study groups. Designation as normal should not be construed as meaning more than conformity with the characteristic level of performance for the average, healthy individual, unobstructed by excessive detrimental influences, congenital or acquired. In determining the absence of excessive detrimental influences two types of investigation were employed.

TABLE 10

GENERAL CHARACTERISTICS OF EXPERIMENTAL SUBJECTS IN STUDY II

Subject	Sex	Chronological age	ANATOMIC AGE				INTELLIGENCE QUOTIENT			Basal metabolic rate	Weight
			Based on Hand			Todd§	Stanford Binet	Arthur Point	Goodenough		
			Todd*	Flory†	Kelly‡						
number		mo.	mo.	mo.	mo.	mo.					kg.
1	M	55	47	34	< 60	49	93	—	109	+4.0	16.90
2	F	63	63	38	—	58	114	112	92	−2.6	16.83
3	M	65	62	51	75	66	100	113	88	−2.6	18.32
4	F	67	75	57	—	68	103	107	—	+1.7	18.06
5	F	71	78	53	—	72	114	96	89	−7.6	19.77
6	F	76	86	72	—	84	115	142	100	−9.9	20.25
7	M	78	85	76	94	84	88	99	75	−8.7	21.55
8	M	83	82	46	77	81	95	98	91	−4.1	20.76
9	M	95	90	67	88	90	96	99	80	−7.3	22.91
10	M	98	122	100	121	122	84	106	—	−16.3	27.60
11	M	104	88	73	106	94	93	140	—	−0.9	23.98

GENERAL CHARACTERISTICS OF EXPERIMENTAL SUBJECTS IN STUDY III

Subject	Sex	Chronological age	ANATOMIC AGE				INTELLIGENCE QUOTIENT			Basal metabolic rate	Weight
			Based on Hand			Todd +	Stanford Binet	Arthur Point	Goodenough		
			Todd*	Flory†	Kelly‡						
number		mo.	mo.	mo.	mo.	mo.					kg.
1	M	103	105	90	111	105	93	—	109	+0.8	27.24
2	F	110	117	101	—	120	114	112	92	+16.1	27.42
3	M	113	121	99	131	123	100	113	88	+12.4	29.37
4	M	126	129	108	122	127	88	99	75	+2.2	31.62
5	M	131	129	102	116	131	95	98	91	−0.9	33.74
6	M	142	141	111	118	144	96	99	80	−0.9	35.68
7	M	146	153	139	176	156	84	106	—	−9.0	41.81

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* Averages of ratings by Todd and Francis according to Todd method.[350]

† Determined by method of Flory.[160]

‡ Determined by method (for boys only) of Kelly.[175]

§ Averages of ratings by Todd and Francis for six age assessments based on roentgenograms of the foot, hand, elbow, knee, shoulder and hip.

+ Ratings by Francis according to Todd method.[350]

(1) Medical, school, and social agency records of each subject were studied for direct evidence of considerations which would exclude the child from the normal group.* (2) Various clinical, physical and chemical procedures were used to establish that the nutritional status of the child was within normal limits. Selection

TABLE 10

GENERAL CHARACTERISTICS OF EXPERIMENTAL SUBJECTS IN STUDY II

ANTHROPOMETRIC MEASUREMENTS (centimeters)

LENGTH				WIDTH					DEPTH	CIRCUMFERENCE				
Recumbent Length		Head	Tibia	Bi-aero-mial	Inter-eristal	Inter-tro-chan-teric	Head	Chest (nip-ple line)	Chest (nip-ple line)	Head	Chest (nip-ple line)	Abdo-men	Upper arm	Thigh
Total	Stem													
105.6	59.9	17.0	22.1	20.4	17.5	18.8	14.5	17.6	13.3	50.2	53.3	50.0	15.0	29.0
103.8	59.1	16.5	21.4	20.2	17.4	19.1	13.6	16.9	13.1	48.3	50.0	49.0	15.6	30.0
113.1	64.2	18.0	22.9	20.5	17.5	18.7	14.7	18.4	12.8	51.7	52.2	50.0	14.2	28.5
108.0	58.5	17.4	22.5	20.6	18.0	19.0	13.5	17.8	13.7	49.9	53.5	52.0	15.8	30.5
114.1	62.3	18.0	24.9	21.1	18.4	20.5	13.6	17.7	14.4	50.3	54.5	52.0	14.7	30.5
116.1	63.4	17.7	24.4	22.1	18.5	19.8	13.5	16.6	13.0	49.6	49.5	49.5	16.0	32.8
117.9	67.0	19.6	24.0	22.1	19.1	20.1	14.3	18.9	14.0	53.6	56.5	55.0	16.0	32.5
118.0	64.2	17.6	25.8	22.3	18.6	19.3	14.5	18.9	14.5	50.6	56.5	51.0	15.4	30.8
118.1	64.8	17.6	25.8	21.9	18.8	20.8	14.7	20.8	15.0	51.4	60.3	55.5	16.8	34.5
135.4	71.7	19.5	30.0	24.2	21.7	24.0	14.2	20.2	14.8	53.3	61.0	54.5	17.1	33.0
124.3	67.6	19.7	27.2	24.5	21.2	21.9	13.8	19.1	14.2	54.2	55.0	56.0	16.2	34.3

GENERAL CHARACTERISTICS OF EXPERIMENTAL SUBJECTS IN STUDY III

ANTHROPOMETRIC MEASUREMENTS (centimeters)

LENGTH				WIDTH				DEPTH	CIRCUMFERENCE				
Recumbent Length		Head	Tibia	Bi-aero-mial	Inter-eristal	Head	Chest (nip-ple line)	Chest (nip-ple line)	Head	Chest (nip-ple line)	Abdo-men	Upper arm	Thigh
Total	Stem												
129.9	70.6	17.6	29.3	24.6	20.2	15.0	20.0	15.7	52.2	64.5	58.5	18.6	36.0
129.1	71.8	16.9	28.7	25.1	20.0	14.3	19.6	14.2	50.5	58.5	56.0	18.3	39.0
139.3	76.8	18.7	30.3	25.6	19.9	15.3	21.2	13.7	53.5	60.5	53.0	16.6	35.3
137.2	76.9	19.6	29.3	25.5	21.6	14.7	21.3	15.8	54.8	64.5	60.5	18.6	39.0
144.0	77.0	18.0	33.5	26.9	21.6	15.1	22.0	16.5	52.4	67.5	59.5	18.8	40.0
136.3	74.8	17.6	30.8	25.0	21.7	15.1	22.9	17.2	52.8	70.5	61.0	20.8	43.1
157.4	82.8	19.2	36.8	28.1	23.6	14.5	23.4	16.6	54.0	70.0	61.5	20.8	41.0

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* The medical histories of children under consideration for the study group were thoroughly reviewed by Charles Wilson, M.D. and Marsh W. Poole, M.D. A majority of the children considered had been "in care" of the Village since infancy and complete, clinic records of periodic examinations were available.

was not confined to physical and nutritional status but considered tissue structure and function, intelligence, and personality.

The complete medical histories of a large number of children were closely inspected and those eliminated which contained evidence of any possible abnormal affecting factor such as hereditary disease or predisposition, congenital or acquired, physical defect, deficiency or idiosyncrasy. From the remaining group those individuals were selected whose dispositions made them amenable to control and these were given complete clinical examinations and were immunized.[*] Fluoroscopic examinations were made and roentgenograms of each child's chest were procured and examined, to establish normality of the heart and lungs.[†] Red blood cell counts, total and differential white blood cell counts and hemoglobin determinations were made and mineral, hematochemical and blood lipid studies were carried out (see Chapter IX). Anthropometric measurements were taken from which nutritional indices were calculated and complete oral examinations, including roentgenograms of the teeth and jaws, were obtained. Postural examinations were made as well as capillary resistence and skin sensitivity tests. Six roentgenograms of each child were made, showing hand, elbow, shoulder, foot, knee, and hip. The final selection of experimental subjects was made from assessments of differential skeletal maturation according to the Todd technique.[‡] In referring to skeletal maturity the average of the six separate assessments has been used. From the films of the hand skeletal age was calculated by the methods of Flory[100] and Kelly.[175] A sufficient number qualified as normal so that alternates were available in other cottages, for replacement if any subject was forced out of the study group by unforeseen contingencies.

Evaluation of Nutritional Status and Growth

A number of the procedures used in determining the normal nutritional status of the experimental subjects were also employed to estimate changes in nutritional status and measure growth. During the studies the children were examined at frequent intervals by the Village pediatrician to eliminate the possibility of any slight illness being overlooked. Total red and white cells and differential counts were obtained two or three times during the studies. Additional blood determinations were made at the time of the last study, and, for some children, counts were procured during the interval between studies. Hematochemical determinations were not possible during the metabolic balance studies, for the blood sampling would represent an appreciable loss of chemical elements from the body.

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[*] The children were given Dick, Kahn, Schick and tuberculin tests. When Dick or Shick tests were positive the children were given, respectively, scarlet fever or diphtheria immunization treatment. Vaginal smears were made for all the girls. The children were immunized against small pox and typhoid fever every two years.

[†] The roentgenological examinations were made by Lawrence Reynolds, M.D., roentgenologist, Harper Hospital, Detroit.

[‡] T. Wingate Todd, M.D., and C. C. Francis, M.D., Department of Anatomy, Western Reserve University Medical School, Cleveland, separately evaluated the differential skeletal maturation[350] (skeletal maturity) of the children. The individual ratings differed by not more than one-half month in any instance; since variations within three months are not significant by the method, the ratings of the two examiners were averaged for the final values.

A study of nutrition and growth is not complete without some knowledge of the development of the dental arches and the occlusion of the teeth[199–201]; of any undesirable habits or physical abnormalities which may influence normal growth and development of the teeth and dental arches[198]; the occurrences and number of teeth present, the condition of the periodontal membranes, and the environmental conditions in the mouth, such as dental caries activity and oral hygiene. Each child's mouth was carefully examined and observations recorded on the condition of gums and investing tissues, occlusion, developmental defects, and enamel formation. Cavities and developmental pits were located by means of a sharp-pointed explorer (Figure 18) and bite-wing roentgenograms were taken to locate incipient interproximal caries not found with the explorer (Figure 19).

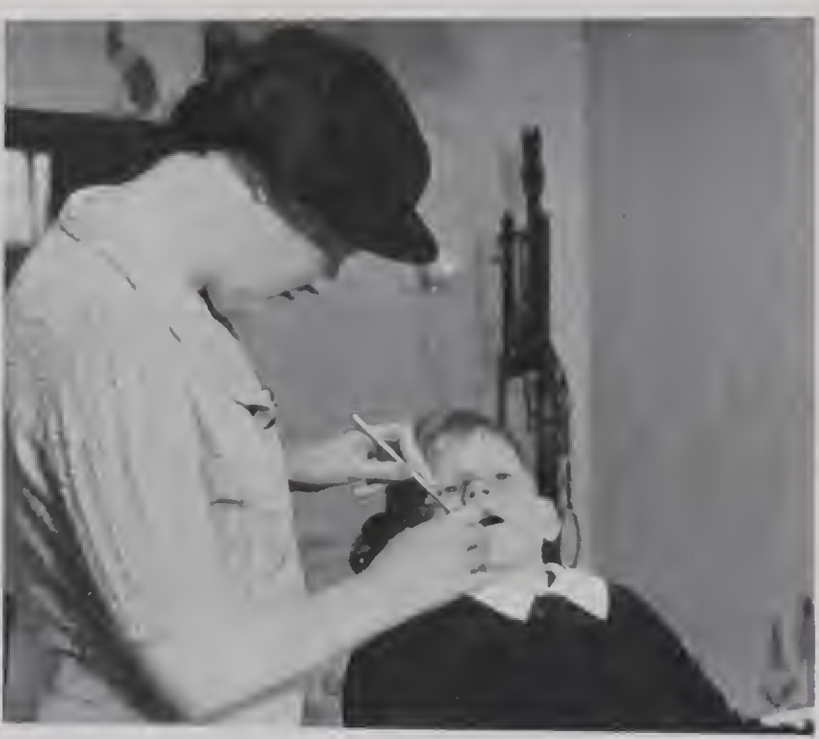

FIGURE 18. Dental examination.

Some of the children had plaster models made of their mouths and roentgenograms (Figure 20) of their jaws,* and dental caries activity was determined by estimation of the relative numbers of lactobacillus acidophilus and other acidogenic organisms in the saliva[321] and by the colorimetric measurement of the acid production of all the organisms of the saliva inoculated in a selective medium.[322] As a result of these examinations and special observations of the oral and dental status, our children were shown to be free from infected teeth and diseased gums (periodontoclasia). Differences were observed in the way the teeth were aligned in their respective arches, but the teeth of all the children were considered to occlude normally. In general, their teeth

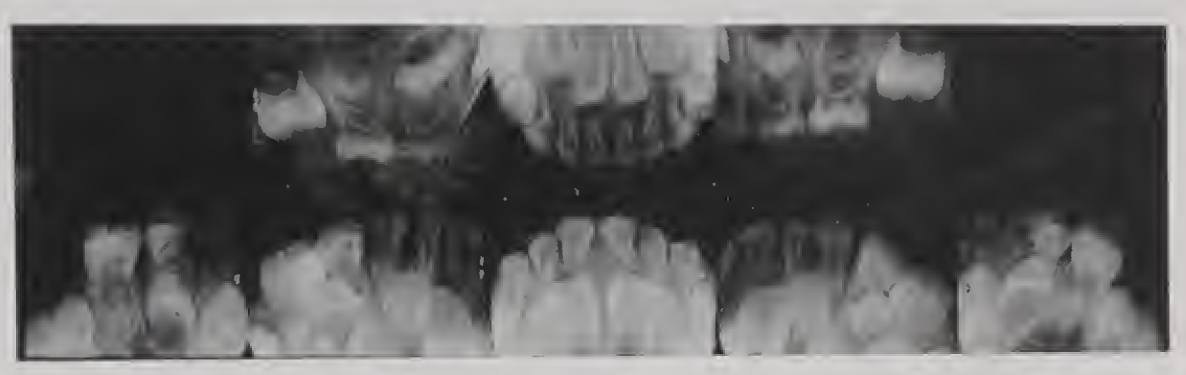

FIGURE 19. Bite-wing roentgenograms.

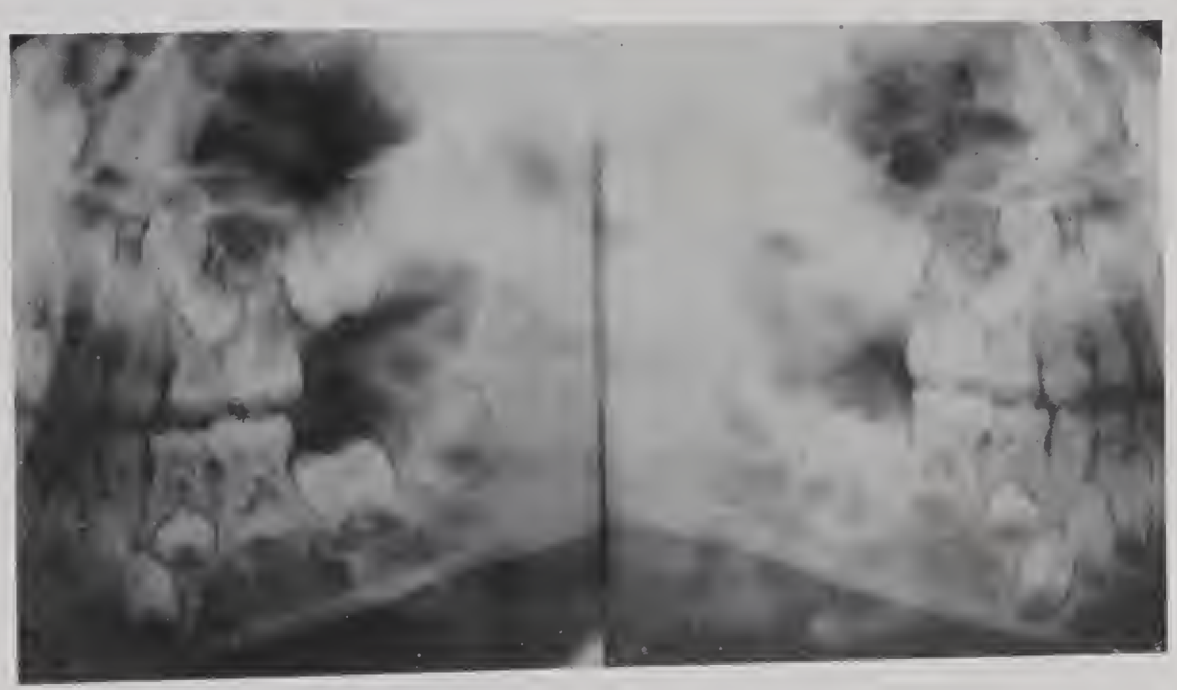

FIGURE 20. Roentgenograms of jaws.

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* Some of the children have served as subjects in the extensive studies of Samuel J. Lewis, D.D.S. on dental arch and facial growth and those of the Dental School of the University of Michigan on dental caries.

were straight, their jaws well-shaped, their deciduous teeth were shed and their permanent teeth erupted at normal ages.

Not all of the children had sound teeth; the number of dental caries lesions, in either temporary or permanent or both sets of teeth, varied from none to several cavities per child; the structure and texture of the teeth[233] varied from hard calcified teeth to those with some degree of irregularly and poorly calcified with developmental pits and open fissures, especially observed in the molars. Oral hygiene was generally good or fair since the children were accustomed to periodic dental examinations and prophylaxis treatments, and dental reparative work had been done when indicated.

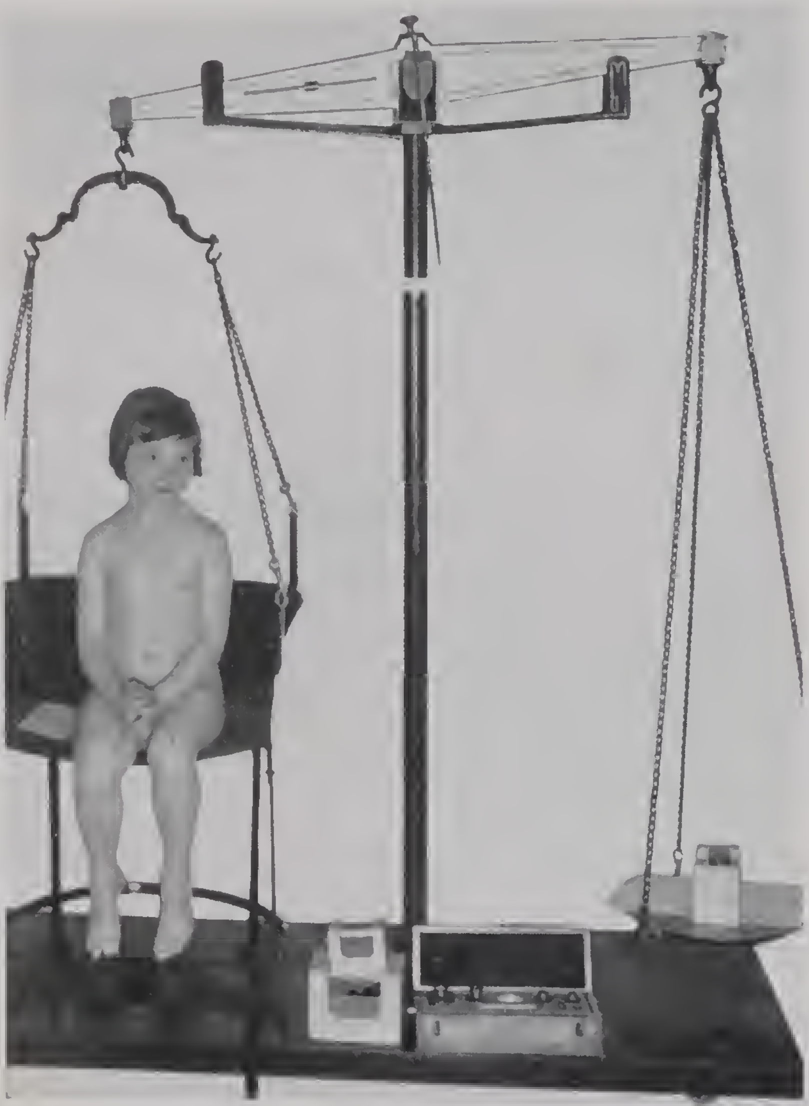

FIGURE 21. Troemmer beam balance.

All infected adenoids and enlarged or infected tonsils were removed prior to initiation of the metabolic study. There were no cases of excessive indulgence in habits known to interfere with the normal action of the tongue, lips or cheek muscles and consequent teeth position and jaw development (finger sucking, lip biting or sucking, tongue and cheek biting). Pertinent to the discussion of nutrition and chemical growth is that there existed no oral, dental or physical irregularities which would interfere with the primary function of the jaws and teeth, satisfactory mastication of the food eaten and thorough mixing of it with the saliva. Since no deterent influences were found in the mouths of the children, we have assumed that all food was adequately prepared for maximum exposure to the digestive enzymes and juices in the stomach and intestinal tract.

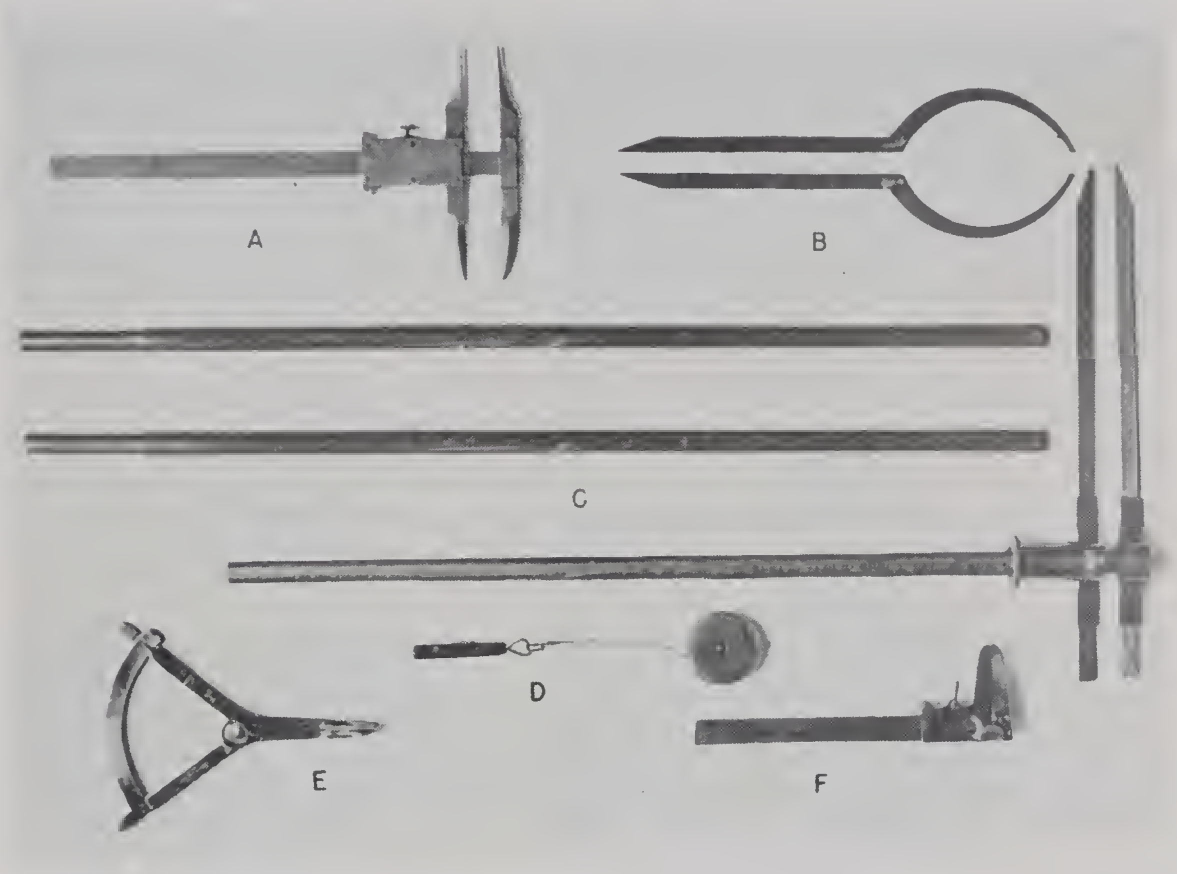

Figure 22. Anthropometric instruments: A. Small sliding calipers with set screw. B. Curved arms to be used in place of the parallel arms of the anthropometer. C. Anthropometer. D. Steel metric tape with Gülick constant-tension, spring handle. E. Fat calipers. F. Small sliding caliper with spring.

The children were weighed every fifth morning throughout the studies upon a Troemmer beam balance, accurate to 0.2 gm. (Figure 21). Weights were taken nude, before breakfast, after urination and, when possible, after defecation. Each weight was taken by two members of the staff and the readings averaged. Measurements of recumbent total and stem length were taken upon a measuring board at fifteen-day intervals, by two technicians. The averages of the duplicate weight and length measurements were plotted and smoothed curves derived. Values from the smoothed curves have been used in all calculations. Anthropometric measurements were made, monthly, and included circumferences of the head, chest, abdomen and upper arm, made with a steel metric tape with a Gülick constant tension, spring handle; and biacromial, intercristal, intertrochanteric widths and tibial length, made with an anthropometer (Figure 22, 23, 24). These measurements were also made at intervals between metabolic investigations. Additional sets of roentgenograms of the bone joints were obtained periodically and full-length exposures were made of their skeletons (see frontispiece).

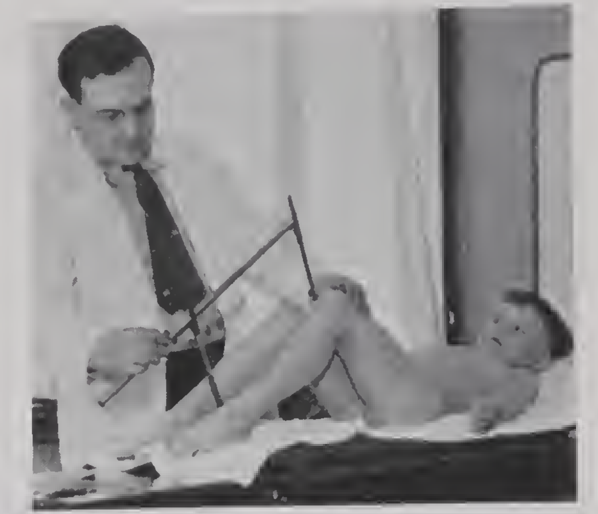

FIGURE 23. Measuring tibial length with anthropometer.

After one metabolic study, the children were established on comparable diets and the responses of their gastrointestinal tracts investigated roentgenographically, during passage of test meals of barium in water, milk, meat, cream and glucose. Later, seven of the same children were subjects of further roentgenological observations; the barium-milk test meals were repeated and the affect of carmine upon gastrointestinal activity was studied. Records were kept of the time required for the carmine markers, given to separate the feces of adjoining balance periods, to be passed through the gastrointestinal tracts of the children and the frequency and wet weights of the defecations were tabulated.

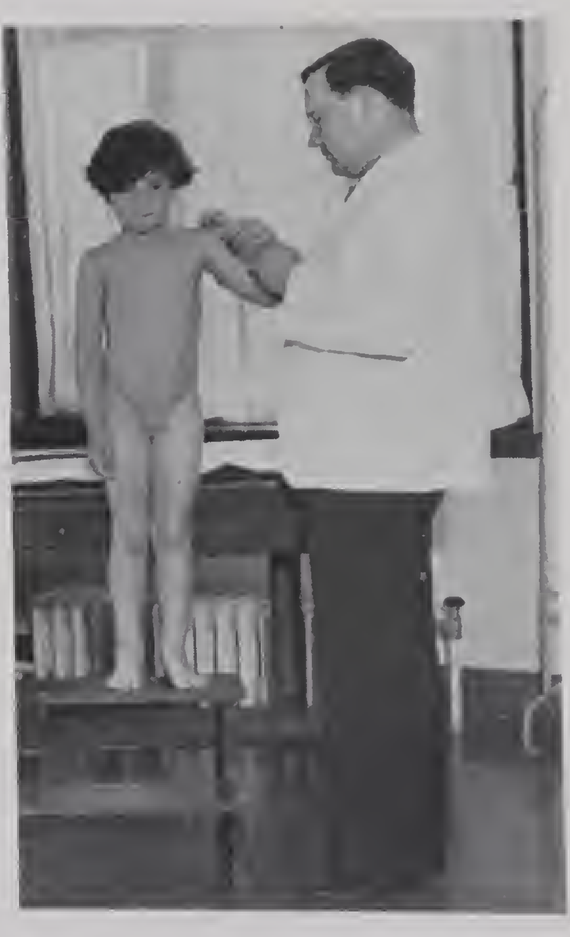

FIGURE 24. Deltoid measurement with small sliding caliper with spring.

In all investigations of human action and reaction, activity and emotional factors are represented in the results. Under environmental conditions as nearly normal as the requirements of the investigation permitted, an attempt was made to determine the relative activity of the different children by fastening pedometers to their belts (at the back) during the weekends, since this was the time of greatest activity. The children were closely watched during these periods to prevent any tampering with the instruments. Records were maintained of any outstanding emotional incidents or reactions on forms (Figure 25), designed so that evaluations by several staff members could be compiled. During the most recent study, a child guidance worker was assigned to the staff at the Village in an attempt to evaluate: (1) the emotional effect of the investigative procedures upon the subjects, and (2) any permanent effect of the procedures upon the emotional patterns of the children.

EMOTIONAL REACTIONS

Date: Name:

Relaxed						Tense
Energetic						Listless
Good-natured						Quarrelsome
Apathetic						Interested
Tired						Rested
Stubborn						Cooperative
Apprehensive						Secure
Calm						Excitable
Dejected						Happy
Affectionate						Indifferent
Easy-going						Irritable

(Details of any emotional upsets)

FIGURE 25. Form for estimating emotional reactions.

The significance of normal posture and the important role it plays in the preservation of health is well recognized. Because posture is intimately related to body functioning, records of changing posture as growth and development proceed are valuable in the assay of nutritional status and physical progress. The recording of natural posture presents many difficulties. Even under the most highly standardized physical conditions there still remain the emotional reactions of the subject to cope with in taking nude pictures. Seldom does a child stand naturally before the camera, nude and trying to stand straight. This consciousness may lead the subject to place his feet in an unnatural weight-bearing position and an artificial placement of the shoulders, abdomen and legs. As part of the health record we obtained postural photographs of the nude subjects according to the method developed at Merrill-Palmer School.[337] Within the limitations of the method, these have contributed pertinent information on the physical status of the children (Figure 26).

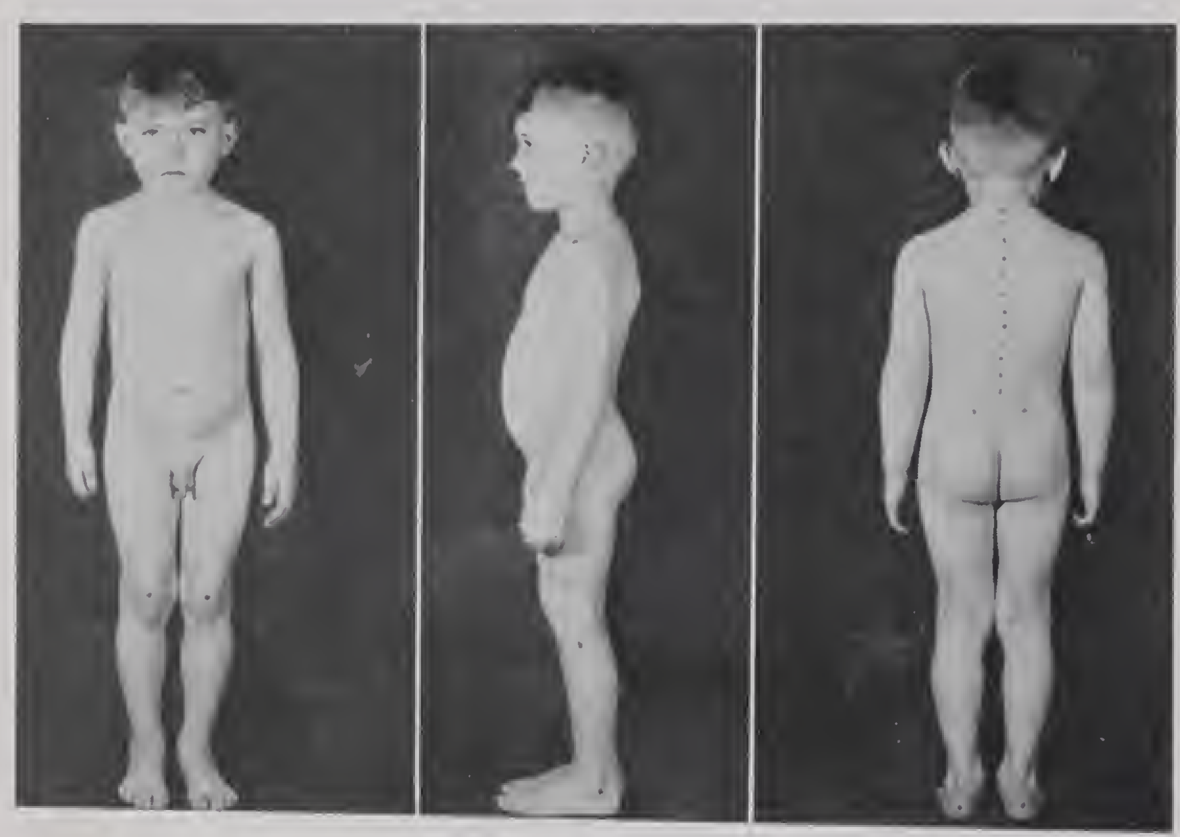

FIGURE 26. Postural photographs.

Periodic impressions of the feet, accurately taken, record the natural position of the feet in walking and standing and are of special value in showing changes that take place in the musculature of the feet and the efficiency of proper footwear and resultant hygiene of the feet of children. In recording and studying the condition of the feet of our children, we used the method developed by King.[178] This method seemed the best means of making a permanent record of the natural standing position of the feet, as well as an accurate record of the position of the feet in walking (Figure 27).

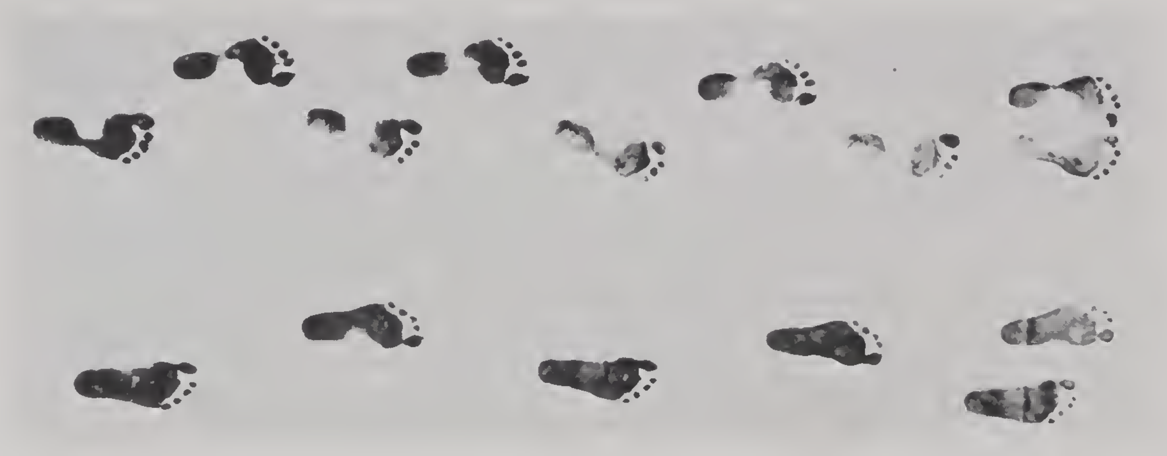

Figure 27. Walking and standing footprints of two children.

The determination of the energy expended by the protoplasmic tissues when all of the modifying factors of activity, food and environmental temperature are producing their minimal effect has become an integral part of assaying the nutritional status of an individual. This determination of the basal or minimum heat production of the energy metabolism of muscles and internal organs of the body includes the involuntary muscular activity of breathing, of the heart in the resting state, and of the smooth muscles of the alimentary canal. The test records the performance of the body at a specific time under a given set of conditions, none of which can be duplicated exactly on other occasions. The relative proportion of active and inactive tissue in the body varies greatly with different individuals and may vary considerably in any one individual at different times, however, basal metabolism tests do provide practical information on the differences in the reactions of the subjects themselves.

Basal metabolic rates were determined for each child every ten days to two weeks,* but only one child's rate was determined any morning. When selection of a subject for the following morning was under discussion, the children begged to have their "breaths taken" and took great pride in making good records.

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* Two and sometimes three ten-minute periods were recorded on each test day. The best six-minute period was chosen as the basal line, and the average of these periods which agreed within five per cent of one another was taken as the basal metabolism figure that day. On the occasional day upon which the measurement did not agree within five per cent, the lowest test was recorded. Pulse rate counted at the wrist for a full minute before and after each test, oral temperature, and dyostolic and systolic blood pressure were also recorded before and during the test periods. Respiration rates were calculated from the kymograph

On the morning of the test the child was carried from his bed to the metabolism room (Figure 28) where he lay in a comfortable bed, adequately covered, for at least a half hour before initiation of the test. The children were unafraid and gave every indication of possessing personal security and nervous stability during the tests, which were made in the post-absorptive state, food having been consumed last the evening before.

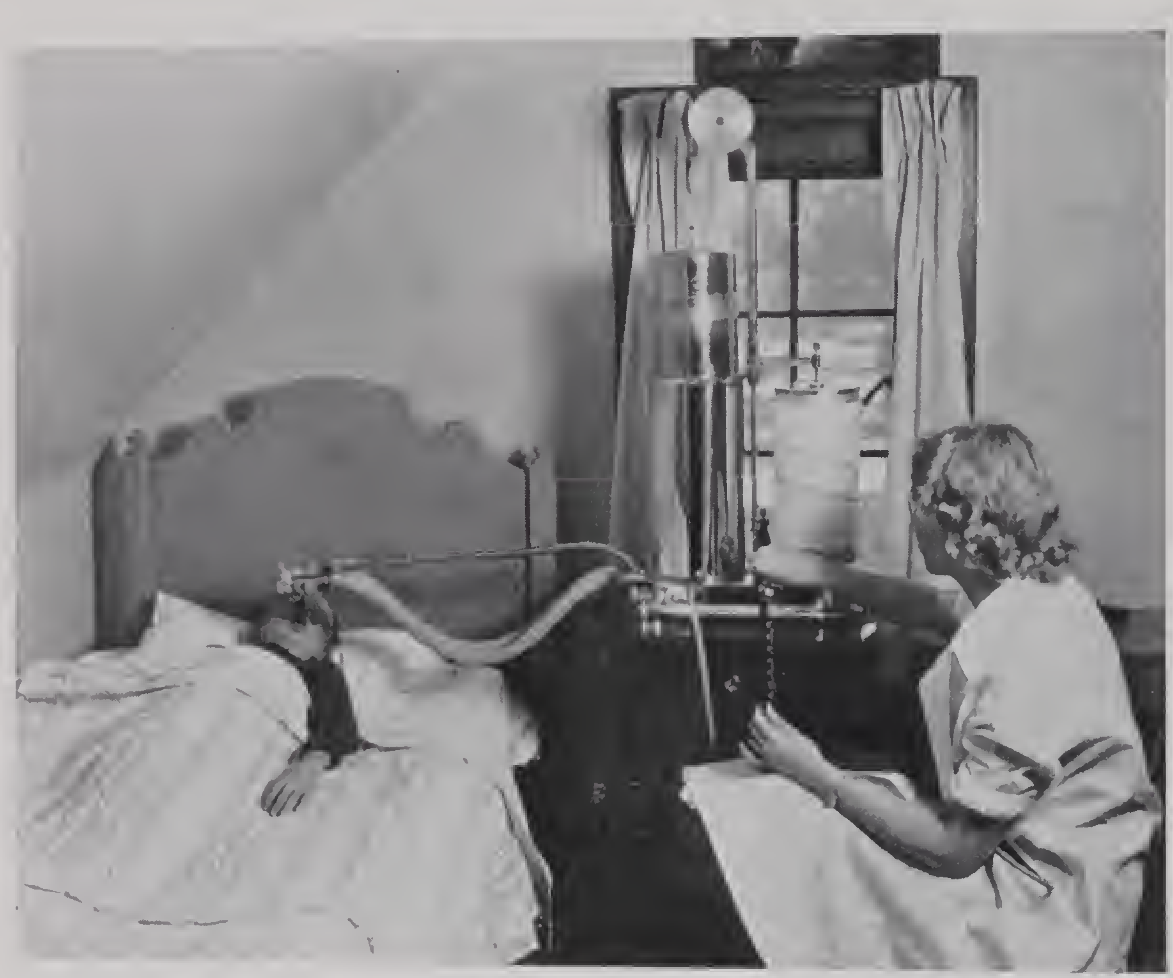

FIGURE 28. Basal metabolism room.

The percentage frequency distribution of 232 basal metabolic rate determinations with 18 children, 4 to 12 years of age, calculated according to Boothby-Sandiford standards[25], is shown in Figure 29. Body temperature, pulse and respiration rates and blood pressure varied slightly for each subject. The range of the averages for the boys and girls in the group are shown in Table 11. Correlation coefficients between the basal heat production

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tracings. Tests were rejected when there was an elevated temperature or pulse rate, coughing, an irregular kymograph record, or evidence of shivering or emotional upset. The room temperature, outside temperature and relative humidity were recorded.

per hour and body temperature, pulse and respiration rates, diastolic and systolic blood pressures and their difference, showed no significant relationships.

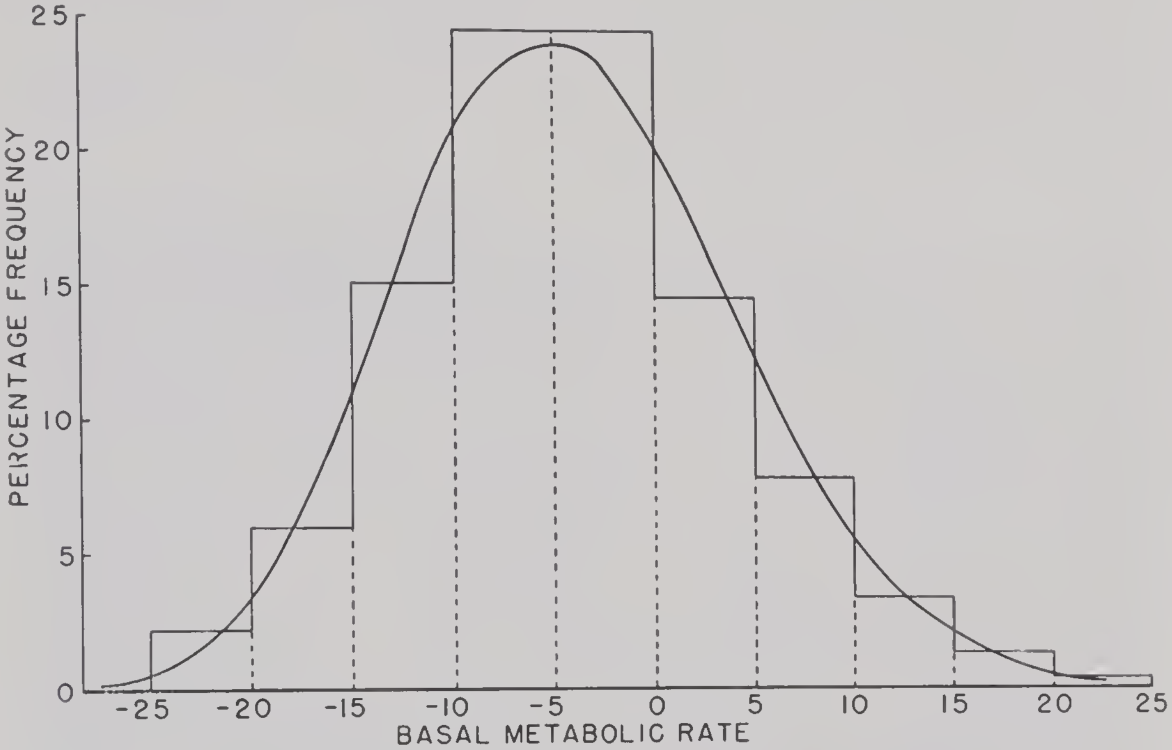

FIGURE 29. Frequency distribution of 232 basal metabolism determinations with 18 children.

Coincident with the determination of basal metabolic rates, records of pulse rate, body temperature, respiration rate and blood pressure were tabulated, and, since extreme variations in temperature and humidity may be influential factors in the prevalence of respiratory disease and intensity of physiological reactions, daily weather reports were obtained for the study periods.

TABLE 11

RANGE OF AVERAGE BODY TEMPERATURE, RESPIRATION AND PULSE RATES AND BLOOD PRESSURE OF 18 CHILDREN

	Boys	Girls	Maximum variation for any child
Body temperature (oral, °F.)	98.2 to 99.0	98.4 to 98.6	1.6
Respiration rate (per minute)	18 to 29	17 to 21	25
Pulse rate (wrist, per minute)	64 to 90	71 to 94	43
Systolic pressure (mm. mercury)	82 to 98	83 to 97	23
Diastolic pressure (mm. mercury)	42 to 70	57 to 67	21
Blood pressure (systolic minus diastolic pressure, mm. mercury)	25 to 48	25 to 37	32

CHAPTER IV

SATISFYING APPETITE AND MEETING RECOMMENDED DIETARY ALLOWANCES

ONE of the essential requisites of continuous nutritional investigations over several weeks or months is the provision of food which will satisfy the demands of hunger, please the subjects, and anticipate the chemical requirements of the body. All the science and art of dietetics must be mustered to keep human beings contented and happy on a restricted experimental regime and, at the same time, meet the changing chemical needs for activity, physical growth and development of the body without hampering social and intellectual development. For the sake of accuracy in biological and chemical results it is necessary to standardize all procedures used in the conduct of a study, in order that variations which are not the result of the subject's physiological activity, may be reduced to a minimum. Growth and development of an organism as a whole implies the continuous, active participation of all the necessary food nutrients and the surrounding environment. It is essential that dietaries be scientifically planned, considering season, environment, the variability of the foods and differences in age, size, activity, sex, race, nutritive state and physiological activity, in order that chemical requirements be met at all times.

Absolute knowledge of the daily food requirements of the human being during his different epochs in life is far from complete. The science of nutrition has made great strides in investigating the processes by which inorganic and organic materials are transformed into human substance but many unsolved dietetic and nutritional problems remain. Thus far, the fundamental facts acquired in large part have been arrived at via systematically planned biological tests on animals, but increased knowledge from trial and error studies of human beings has brought realization that observations on man himself constitute the only true measure of human growth and nutritional performance. The changing chemical needs of a child can be met only by a dietary consisting of sufficient quantities of all the materials essential for physiological maintenance and construction of body tissues. Diets not only must contain ample quantities of the known cardinal nutriments, but also these nutriments must be in the forms and relative proportions to one another which provide for unquestionable availability, digestibility and absorbability. Beyond the culinary value of the foods, it is paramount that they be liked and well tolerated by each subject. Each year additional evidence is presented which shows the wide prevalence of food idiosyncrasies.

Preliminary to each of our own studies, the existing dietary standards were diligently studied for the purpose of anticipating the chemical requirements of average healthy children between the ages of 4 and 12 years. Accordingly, each possible candidate was subjected, for two or three weeks, to a dietary carefully planned, prepared and served. At the same time, the accurate regulation of habits and restriction of routine necessary in metabolic balance investigations were imposed, to determine the individual's adaptability to more prolonged observations. During this preliminary period dietary standards were put to the practical test of satisfying hunger and the aesthetic tastes of the child. The acumen and dispatch with which all essential details were adhered to by the child were observed, in the interest of future accuracy and expediency in obtaining scientific facts. During the preliminary observation, dietary adjustments were made to meet the particular needs of each child and all non-cooperative dawdlers and unfit subjects were eliminated without great loss of time, effort and expense.

Distribution of the Total Dietary

In considering diets as sources of essential substances for growth, properly distributed, it is prudent to give a liberal and well-balanced mixture. Some proteins are notably deficient of certain essential amino acids; the body may be able to synthesize some of these but others must be present as such in the diet; certain of these are more abundant in animal protein and others in vegetable protein. It is necessary, therefore, to choose wisely between animal and vegetable foods. In dietetic practice the proteins of milk, cheese, eggs and meat have been used to furnish the known essential amino acids for growth, and to supplement the incomplete proteins of cereals and vegetables.

A diet may yield sufficient energy to meet the requirements of the organism, the protein may be sufficient in quantity and high in nutritive value, available inorganic salts may be present in desirable amounts and proportions to one another, but if a synergistic mixture of vitamins is not present in proper quantity, nutritive failure will result. They are synthesized by plant life and transferred to the human body in plant foods, directly, or indirectly, by way of animal food. Green vegetables, fresh fruit and milk have been termed "protective foods" by McCollum because they furnish vitamins and mineral salts in the most desirable forms.

No two natural foods are exactly alike in caloric value or in mineral, nitrogen, amino acid, and vitamin contents. Although there are differences in the composition, digestibility and utilizability of foods, many foods are sufficiently alike in general nutritive characteristics to be grouped together for practical dietetic purposes. The guiding principle in practical selection of an adequate diet, therefore, has been to choose from the following classes of foods: cereals, fruits and vegetables, dairy products, meats and sugars. By the use of this grouping of foodstuffs it is possible to test the adequacy of any diet by classifying the amounts and proportions of the constituent foods. (Figure 30)

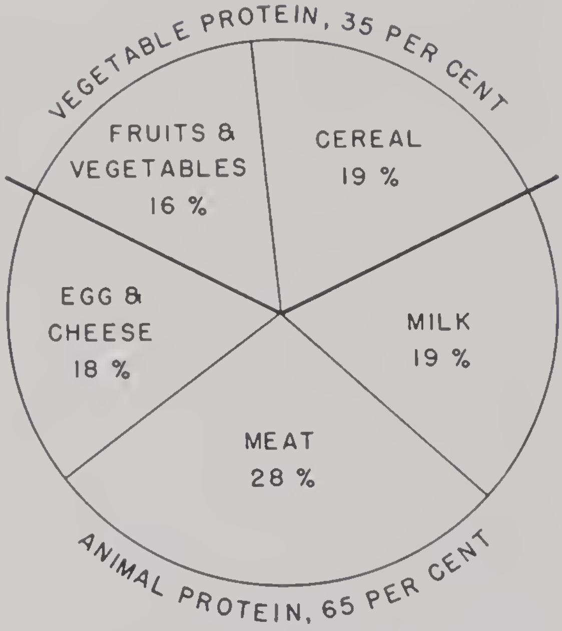

FIGURE 30. Protein distribution of dietary.

Evaluation of Dietaries

There may be considerable differences between energy value of a diet as determined by calculation from a standard table of food composition, the usual dietetic practice, and energy value determined by calorimetry or by chemical analysis of the diet for protein, fat and carbohydrate (Table 12). Evaluation of the plane of nutrition provided by a diet depends upon: (*a*) which fuel value factors have been used in making the calculations; (*b*) what allowances, if any, have been made for waste in preparation and losses in digestion; (*c*) if the heat of combustion has been used, whether any allowances have been made for losses in urine and feces. Investigators often fail to record the precise methods used in determining the fuel value of the diet under consideration. Morey[240] recently emphasized this deficiency.

TABLE 12

CALCULATED AND DETERMINED PROTEIN, FAT AND CARBOHYDRATE CONTENTS OF DIET OF A 12 YEAR OLD BOY

Values in grams

	Protein		Fat		Carbohydrate	
	Calculated*	Determined†	Calculated*	Determined	Calculated*	Determined‡
Cereal	14.4	15.1	5.3	3.2	101.8	105.2
Butter, milk and cheese	22.2	19.7	86.0	81.2	24.8	30.7
Animal products	35.2	33.2	19.5	19.8	0.7	2.7
Fruits and vegetables	13.5	13.2	9.2	8.1	101.8	92.9
Sugar and honey	0.1	—	—	—	34.2	34.7
Total	85.4	81.2	120.0	112.3	263.3	266.2

* Using Sherman[309] standards for individual foods.
† Analytical values for nitrogen ×6.25.
‡ Dry weight of intake minus weight of protein, fat, and minerals.

To illustrate the importance of considering the various methods of determining the fuel value of a day's food supply we have selected the diets of two boys of different ages and body build (Table 13). Protein of animal origin composed 65 per cent of the dietary protein; 35 per cent was from vegetable sources (Figure 30). Of the total caloric intakes, 49 and 54 per cent were obtained from animal sources for the 9 and 12 year old boys, A and B, respectively. Rubner found an 8 to 10 per cent energy loss in the process of digestion.[206] In the two boys the loss through the urine was 4.0 per cent of the determined caloric intake and 5.4 and 4.4 per cent was lost in the feces, totalling 9.4 and 8.4 per cent loss in digestion. Results of all three methods of calculating heat of combustion of the intake and the physiologic fuel value, conform with Rubner's estimate of 8 to 10 per cent loss during digestion. Application of the heat of combustion factors (5.65, 9.45, 4.1) to chemically determined amounts of protein, fat and carbohydrate in the diets, resulted in values 4 per cent higher than obtained by calorimetry; the same factors used with published values for the protein, fat and carbohydrate contents of the individual foods, may yield results 8 per cent higher than calorimeter values.

TABLE 13

COMPARISON OF METHODS OF DETERMINING THE ENERGY VALUE OF DIETS*

Values in Calories per day

	CHILD A			CHILD B		
	Calorimetric determination	Chemical determination†	Calculated value	Calorimetric determination	Chemical determination†	Calculated value
Heat of combustion	2187‡	2277§	2362#	2500‡	2605§	2694#
Physiological fuel value	1980‡	2080+	2157+	2289‡	2391+	2473+

* Methods of determining the energy value of diets are compared by applying the methods to the diets given to two boys during a period of 55 consecutive days—Child A, 9 years old; Child B, 12 years old.

† Chemical determination of protein, fat, and carbohydrate in the diets. Protein was determined by applying the factor 6.25 to the results of nitrogen analyses and carbohydrate was determined by difference: dry weight of entire diet minus (weight of protein, fat, and minerals).

‡ Oxygen bomb calorimeter determination. By this method the heat of combustion of the diet includes 5.65 Calories per gram protein, however, by applying the same method of determination to urine and feces and subtracting their heat of combustion from that of the intake, a measurement may be obtained of the physiological fuel value (net Calories) of a specified diet to definite individuals for a given interval.

§ Factors for heat of combustion of protein, fat and carbohydrate, 5.65, 9.45, and 4.1, respectively, applied to chemically determined protein, fat, and carbohydrate contents of the diet.

# Calculated by applying factors 5.65, 9.45 and 4.1 for heat of combustion of protein, fat and carbohydrate, respectively, to the amounts of each constituent in the individual foods as given by the tables of Sherman.[309]

+ Factors (Atwater-Bryant) for physiological fuel value of protein, fat, and carbohydrate, 4, 9, and 4, respectively, applied to protein, fat, and carbohydrate contents of diet.

For 18 children studied over 2440 days the average calorie loss was 3.6 and 4.2 per cent through the urine and feces, respectively, totalling a 7.8 per cent loss of the caloric intake as compared to Rubner's 8 to 10 per cent.

Sampling Techniques and Methods of Analysis: Besides the discrepancies owing to the methods used in reporting energy values, variations in food composition may have a significant effect upon the interpretation of energy intake and utilization. Errors may also arise from the techniques of weighing, sampling and analyzing foods (p. 235). In our investigations each foodstuff subject to variability was sampled daily and the samples combined into a composite for analytical procedures. For comparison, the individual foods were sampled at intervals during the study.

Much smaller deviations were found between different samples of individual foods in our diets than are indicated by the various analyses compiled from the literature by Word and Wakeham.[393] There can be little doubt but that the control measures used in securing the foods were responsible for this greater constancy.

TABLE 14

COMPOSITION OF DAILY FOOD INTAKE GIVEN 12 YEAR OLD BOY DURING 55 CONSECUTIVE DAYS

	Weight as eaten	Heat of combustion	Protein†	Fat	Carbon	CARBOHYDRATE		POSITIVE MINERALS				NEGATIVE MINERALS		
						Total	Complex	Ca	Mg	Na	K	P	Cl	S
	gm.	Cal.	gm.	gm.	gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Cereal														
Corn Flakes	30	115	2.14	0.08	11.4	25.3	0.31	2	11	334	30	12	490	38
Bread, white	50	145	4.88	0.68	13.8	26.1	0.28	30	14	300	54	57	422	61
Bread, whole wheat	50	148	5.54	0.87	14.2	26.9	1.41	32	34	300	102	108	445	68
Crackers, Graham	36	134	2.53	1.56	13.0	26.9	0.62	16	22	242	138	56	186	44
Milk	500	351	15.88	17.18	31.1	27.6		590	50	262	736	449	531	177
Fruits and Vegetables														
Apple	100	55	0.25	0.30	5.4	13.8	0.91	5	6	4	131	13	3	6
Banana	150	129	1.55	0.24	13.0	32.4	0.93	10	50	15	628	49	124	16
Orange juice	100	40	1.04	—	5.2	11.3	—	12	12	4	192	24	6	6
Tomato juice	60	12	0.50		1.3	2.4	0.12	2	6	153	135	10	232	8
Cabbage	25	6	0.33	0.03	0.6	1.2	0.26	11	3	6	40	4		16
Carrots	50	22	0.31	0.08	2.1	4.9	0.70	13	9	29	160	15	57	10
Lettuce	25	6	0.21		0.5	1.0	0.18	4	3	4	43	5		3
Peas (frozen)	25	24	1.71	0.11	2.4	3.5	0.71	6	8	24	74	28	28	11
Potato	120	91	2.63	0.12	9.3	20.1	0.44	10	26	31	469	73	40	52
Peanut butter	16	101	4.64	7.23	7.8	2.3	0.18	4	28	62	111	54	98	37
Meat														
Beef (ground, lean)	100	237	19.63	12.43	22.0	—		10	13	70	290	148	63	164
Eggs and cheese														
Eggs	100	146	10.61	7.32	12.8	2.7		42	2	128	120	165	140	157
Cheese	20	70	3.81	3.98	6.0	3.1		113	3	287	51	175	224	36
Miscellaneous														
Gelatin	3	14	3.00		1.3	—							2	14
Butter	60	540		60.00	41.4	—							2	3
Honey	15	44			4.3	12.4								
Sugar*	22	89				22.3								
Salt	2					—				787			1213	
Water*	557					—								
Total	2216	2519	81.19	112.21	218.9	266.2	7.05	912	300	3042	3504	1445	4306	927
Total calculated‡		2474	85.44	119.91		263.3		1040	347	1860	3684	1638	2866	911

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* Average values for the 55 days. Sugar and water intakes were allowed to vary, within reasonable limits, according to the inclinations of the child, but the daily intakes were recorded.

† Conversion factor of protein from nitrogen = 6.25.

‡ Calculated from tables of Sherman[39] and Sherman and Lanford.[308] Only calcium and phosphorus data are given by these authors for corn flakes, graham crackers and whole wheat bread.

A comparison of the averages of analyses of the food composites (including the individual values for milk, potato, butter and salt) with the sum of the average values for all the individual foods analyzed separately, is given in Table 15. That the two methods of determining the composition of the diets agree so closely is, without doubt, attributable to the care exercised in the selection and securing of supplies; manipulation, handling, serving and sampling of the food; the coordinated efforts of staff and children at the cottages; and the precautions employed by the chemists in the sampling, storing and analyzing of the food specimens at the laboratory.

TABLE 15

COMPARISON OF AVERAGES OF ANALYSES OF FOOD COMPOSITES AND SUM OF AVERAGE ANALYSES OF INDIVIDUAL FOODS* IN THE DAILY DIET

	Basal composite†	Milk	Potato	Salt	Butter	Total‡	Sum of individual foods‡
Heat of combustion, Cal.	1428	351	91		540	2410	2430‡
Protein, gm.	64.44	15.88	2.63			82.95	81.19
Fat, gm.	35.52	17.18	0.12		60.00	112.82	112.21
Carbon, gm.	133.4	31.10	9.30		41.40	215.20	218.90
Positive Minerals							
Calcium, mg.	346	590	10			946	912
Magnesium, mg.	228	50	26			304	300
Sodium, mg.	1927	262	31	787		3007	3042
Potassium, mg.	2282	736	469			3487	3504
Negative Minerals							
Phosphorus, mg.	979	449	73			1501	1445
Chlorine, mg.	2575	531	40	1213		4359	4306
Sulfur, mg.	770	177	52			999	927

* See Table 14 for foods included in the diet and analyses of individual foods.

† Basal composite did not include salt, sugar, butter, potato or milk; potato and milk were analyzed separately and are added to basal for total. The butter oil used was free of minerals.

‡ Exclusive of sugar.

Preparation and Serving Dietaries: While ingesting diets of the same foods and with distributions similar to that shown in Figure 30, normal children grew in size, which was shown by anthropometric measurements and their retentions of nitrogen and the positive (calcium, magnesium, sodium and potassium) and negative (phosphorus, sulfur and chlorine) minerals; they maintained excellent health; bowel movements were regular and satisfactory; they were alert and made suitable advances in their studies; and, their physical performances were as good or better than those of the average American child.

The foods chosen for the studies are among those most frequently recommended for the dietaries of children. They are, usually, well-liked, well-tolerated, economical and available the year around, and the group permits varied and attractive preparation and service (Figure 31). To provide ample stimulation and exercise of the gums, the muscles of mastication, and the periodental membranes, raw carrots, cabbage and apples, and toast were given frequently. It is alleged that raw fruits and vegetables have additional value in that they have an abrasive cleansing action on the teeth.

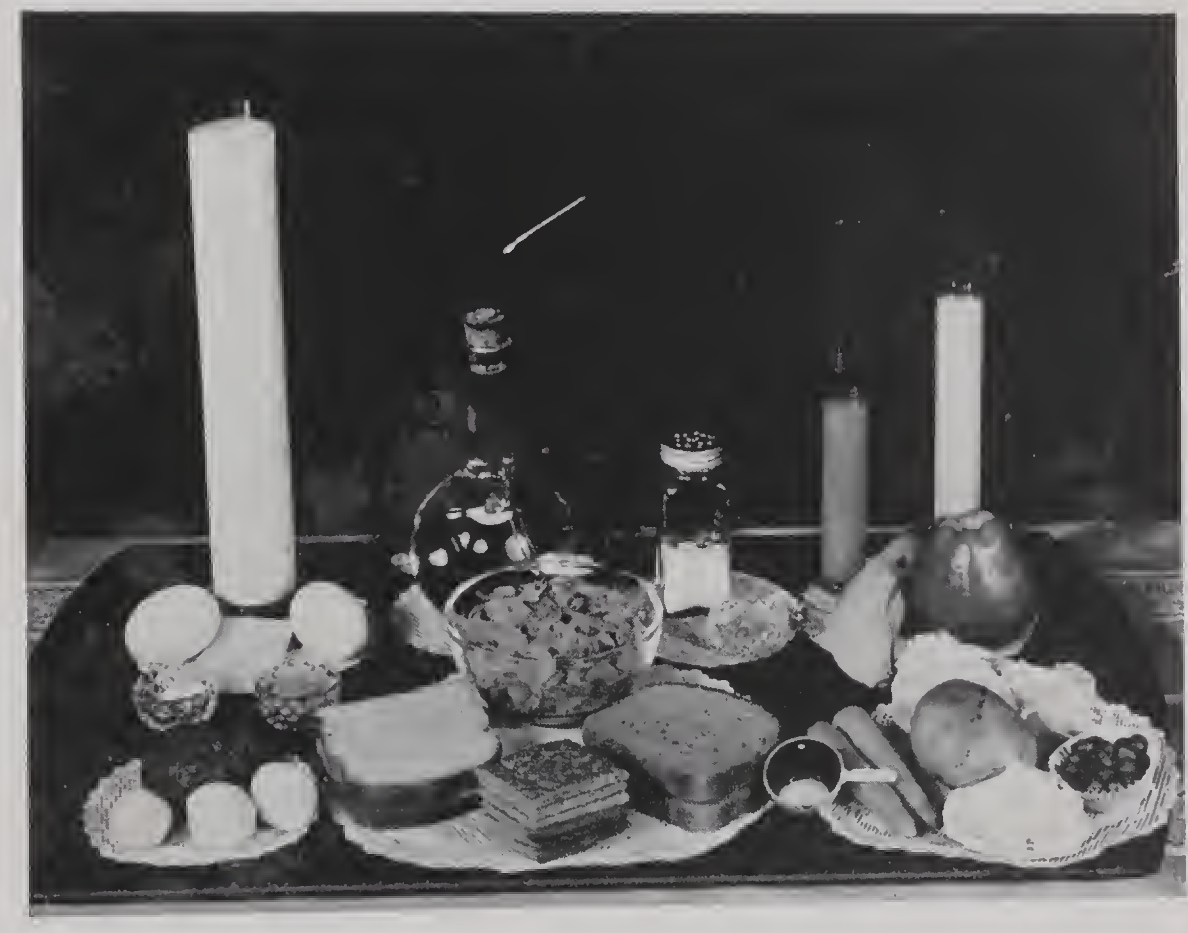

FIGURE 31. Foods in the daily dietary (Table 14) of a 12 year old boy.

Many procedures were employed in keeping the content of the foods as constant as possible. Staple foods were purchased in the largest possible quantities practical at one time, so that inherent variations in composition, owing to variety, season, locality of growth, time of marketing, and preserving, might be minimized. The same types of fruits and vegetables were purchased from the same sources throughout the study, so that all of the apples, cabbage, and potatoes were not only of the same variety but also were grown in the same soil. Bananas of the same variety were selected by an experienced wholesaler and delivered to the Village twice weekly, so that they would be eaten at approximately the same stage of ripeness. One butcher provided lean beef of the same cut and a single creamery provided all milk, controlling the supply so that it came from the same herd of cows and was subjected to analogous pasteurization treatment. Milk, for a five-day period was collected from a single day's batch and held in highly

TABLE 16

CONTROL OF VARIATIONS IN FOOD COMPOSITION OWING TO VARIETY, SOURCE, HARVESTING AND MARKETING

FOOD	CONTROL
Canned orange and tomato juice, peanut butter	Supply for entire study purchased with same lot number stamped into end of cans.
Fresh orange juice	Squeezed with glass equipment and held under refrigeration.
Corn flakes, graham crackers	Same brand and grade purchased in amounts sufficient for three or four weeks.
Apples (Michigan)	Supply purchased for entire study from a single orchard.
Potatoes (Michigan)	Supply for entire study purchased from same harvest, and locality.
Banana	Selected from one shipment by experienced warehouse man so that they would be used at approximately the same stage of ripeness.
Cabbage, carrots (Michigan)	Purchased as needed from a cooperative retailer.
Lettuce (California)	Purchased as needed from a cooperative retailer.
Peas (quick frozen)	Same brand purchased daily.
Honey	Purchased from same apiary.
Cheese, processed American	Weekly supplies of same brand.
Bread, white and whole wheat	Purchased daily from one bakery.
Beef	Same shoulder cuts from same grade beef purchased daily.
Butter	Sweet butter was prepared in 100-pound lots from single tank of milk, frozen and held in cold storage until delivered.
Milk	All milk from same herd of cows, subjected to rigidly controlled methods of collecting and processing. Sufficient pasteurized and irradiated milk for each period was taken from one tank and held under refrigeration for daily deliveries.
Eggs	Eggs from the same flock of hens were purchased from nearby farm.
Gelatin, salt	Chemically pure NaCl and purified powdered gelatin were obtained from a chemical manufacturer.
Water	Distilled water was used from five-gallon bottles.
Sugar	Purchased from same refiner.

controlled refrigeration, only a day's supply being delivered at a time. Processed cheese, sweet butter prepared in large quantities and tomato juice of the same lot number were used. Eggs were obtained from the same flock of hens. Orange juice for a five-day balance period was squeezed with glass utensils* and kept in the refrigerator in small bottles containing one day's supply, tightly sealed to avoid deterioration of vitamin content. The control measures employed are itemized in Table 16.

Only a few of the foods used required special preparation before weighing. The carrots were scraped, potatoes peeled, apples cored, the frozen peas thawed, and the outside leaves removed from cabbage and lettuce, after which the vegetables were washed in distilled water and transferred to absorbent linen towels to remove excess liquid.

It is convenient to weigh each child's total daily allotment of some foods into suitable containers and use the entire amount in each container in the preparation of the day's meals. Even with three workers to prepare food and handle collections, at times during the day everyone is heavily taxed to carry on the necessary routine for ten children. The most pressing period occurs throughout the morning, when the staff must prepare breakfast, attend to collections, weighing, measuring, determine basal metabolic rates, then prepare the noonday dinner. It is almost a requirement that much of the weighing be completed the preceding evening. Staple foods—salt, sugar, bread, graham crackers, butter, peanut butter, cheese, honey, corn flakes—can be weighed for the following day. Salt may be weighed into labeled glassine bags and sugar into jars with perforated tops, which are handy for use throughout the day. A muffin tin, with each section clearly labeled, is suitable for the butter portions and makes a convenient unit to handle in the food preparation. Each child's daily rations of bread and graham crackers can be weighed and kept in marked, individual, wax paper packages, readily available throughout the day. Small waxed paper cups are useful as storage receptacles for the daily allotments of honey, cheese, and peanut butter. Corn flakes are readily weighed into covered jars.

Most of the foods needed to prepare breakfast are those which may be weighed out the night before, but, if eggs are to be served, the day's quota for each child may be weighed before the morning meal. To provide the same weight of egg each day, whole eggs sufficient for all the children may be beaten together and each child's portion weighed from the mixture. Occasionally, for variety, single eggs were weighed and the total intake of egg adjusted by addition from a combined supply. The daily quotas of all other foods may be weighed, individually, after breakfast and placed in separate, marked, covered glass jars which are kept in the refrigerator throughout the day. Weighing the total allotments of solid foods for the day simplifies the routine of carrying on a study and eliminates the weighing errors which would be inherent in weighing the small amounts of each food for each meal. The daily allotment of drinking water (distilled), measured with graduated cylinders into pyrex flasks equipped with glass drinking tubes, proves convenient and novel in diverting attention from the flat taste of the purified water. At each meal, the prescribed amounts of milk, tomato juice or orange juice are weighed into the individuals' glasses, or into the proper dishes if they are to be used in cooking (Figure 32).

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* In an investigation of the growth of infants, orange and lemon juice were found to lose up to fifty per cent of their ascorbic acid content through the use of iron utensils (the iron acting as catalyst in oxidation).

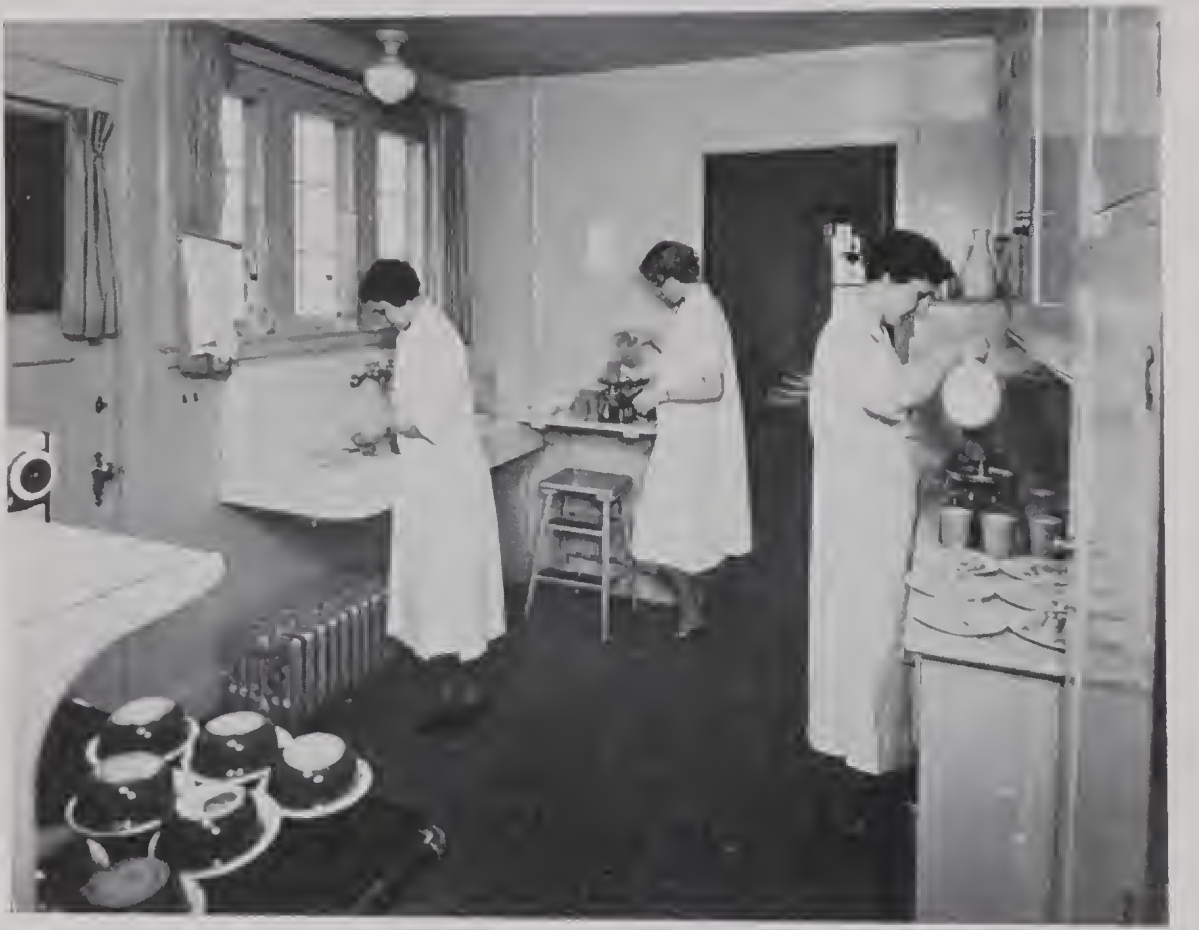

FIGURE 32. Preparation of meal for subjects of metabolic balance study.

Foods were taken from each child's ration for the day and prepared in the combinations required by menus carefully designed to present the meals in the most attractive, appetizing and varied manner possible. Foods to be cooked were placed in individual pottery or porcelain dishes permanently identified with the child's name, and seasoned with butter, salt or sugar from the child's daily allotment. Distilled water was employed for cooking as well as drinking purposes. Care was taken to cook off all excess water and food was cooked and served in the same dish, avoiding any transference losses. Each meal was served to the children at a table, on attractive individual trays so that if any accidents or spilling occurred the food could be recovered and reweighed with minimum error.

Efforts expended in planning varied and appetizing menus with the identical foods used day after day are well rewarded in metabolic studies, especially those involving children (Figures 33, 34, 35). During our studies, complaints about the meals were seldom heard, in fact, the children soon acquired a great deal of interest in the weighing and preparation processes. This interest heightened their appreciation of the necessity of eating all of their food and stimulated caution to avoid spilling. The children vied with each other in scraping their dishes "cleanest" and saved a piece of bread during meals so that they could take up

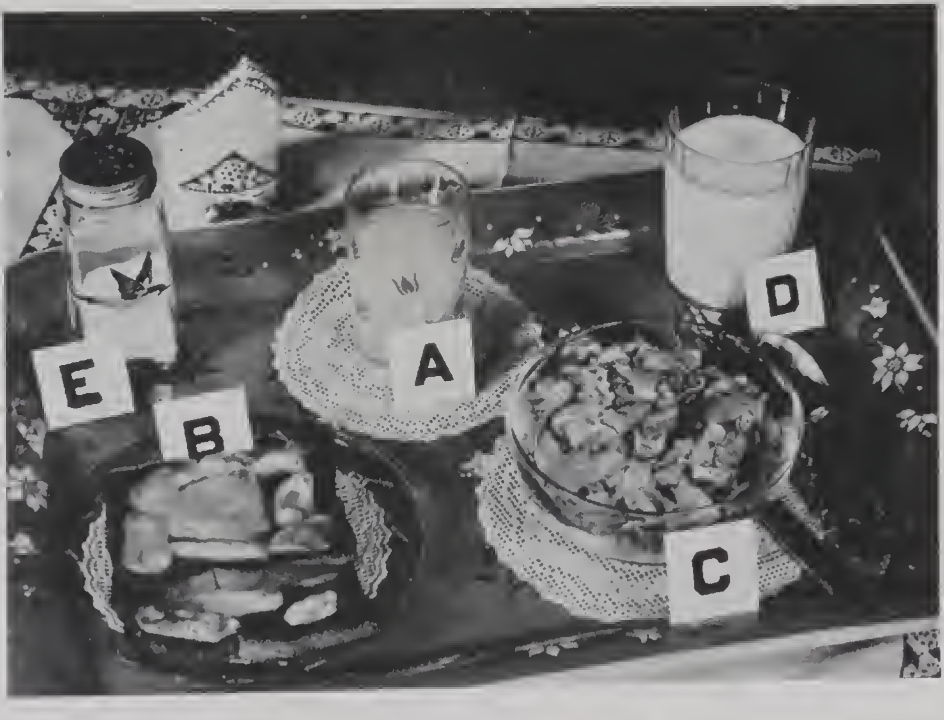

FIGURE 33. Menu prepared from foods listed in Table 14 and shown in Figure 31. Breakfast: A. Orange juice (100 gm.) B. Toast, white bread (50 gm.) with melted cheese (20 gm.) C. Cornflakes (30 gm.) D. Milk (200 gm.) E. Sugar.

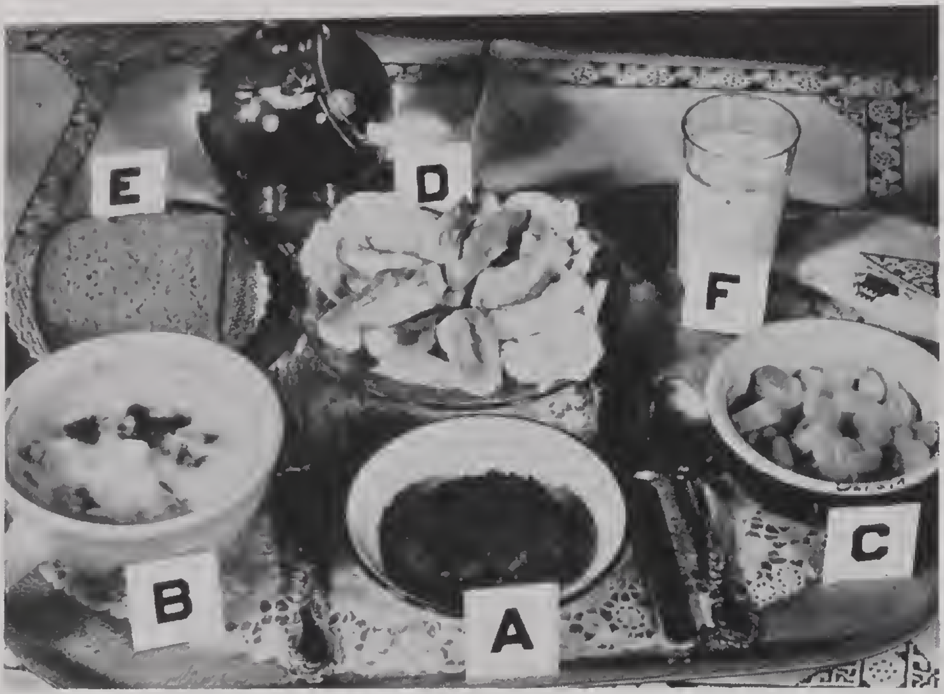

Figure 34. Menu prepared from foods listed in Table 14 and shown in Figure 31. Dinner: A. Beef pattie[1] (100 gm.) B. Escalloped potatoes[1,2] (potatoes 120 gm., milk 50 gm.) C. Buttered carrots[1,2] (50 gm.) D. Banana (75 gm.), peanut butter (16 gm.) cabbage (25 gm.) salad E. Bread,[2] whole wheat (25 gm.) F. Milk (100 gm.).

[1] Salt from daily allotment of 2 gm.
[2] Butter from daily allotment of 60 gm.

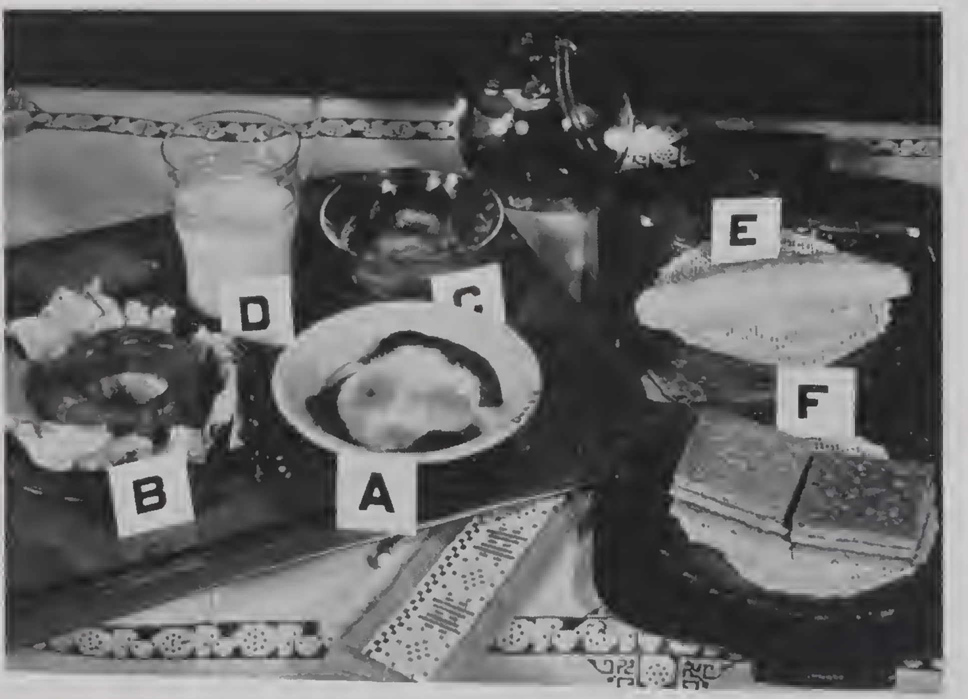

Figure 35. Menu prepared from foods listed in Table 14 and shown in Figure 31. Supper and afternoon lunch: A. Baked eggs (100 gm.) on whole wheat toast[1,2] (25 gm.) B. Aspic salad[2]—tomato juice (60 gm.), peas (25 gm.), gelatin (3 gm.), lettuce (25 gm.) C. Baked apple (100 gm.) D. Milk (150 gm.) E. Banana (75 gm.) F. Graham crackers (36 gm.) with honey (15 gm.).

[1] Remainder of butter from daily allotment.
[2] Remainder of salt from daily allotment.

the small amounts of liquids which spoons could not remove from their dishes.

In planning menus the children struggled with the problems of employing foods in the required amounts and distributing them among the meals to finish with the correct total daily intake (Table 17). They maintained such interest that a competitive spirit arose among them as to who could plan the best day's menu, and thus each meal was met with eagerness. The children preferred their food served in simple forms: plain hamburger pattie, mashed potato, uncooked fruits, and vegetables. The variety of ways in which the foods were prepared satisfied both hunger and aesthetic tastes (Table 18).

At the close of the longest observation period (225 days), when the children were notified that the study was completed and that they were to go on the regular diet of the other children in the Village and could have anything they wanted to eat, of their own accord they chose those foods to which they had become ac-

TABLE 17

DAILY MENUS PLANNED BY CHILDREN*

	MENU I	MENU II
Breakfast	Corn flakes (30) with honey (15) Tomato juice (60) Toast—white (50) with melted cheese (20) Milk (200)	Corn flakes (30) Toast—white (50) with melted cheese (20) Sliced banana (75) with orange juice (100) Milk (200)
Dinner	Mashed potato (120) Apple (100) stuffed with beef (100) gelatin (3) Banana (75) and peanut butter (16) salad on cabbage (25) Graham crackers (18), buttered Milk (150) Bread—whole wheat (25)	Hot beef (100) sandwich, whole wheat (25) gelatin (3) Fried potato (120) Carrot (50) and peanut butter (16) salad on cabbage (25) Egg (25) nog; milk (100)
Afternoon Lunch	Graham crackers (18), buttered Banana (75)	Apple (50) Graham cracker (36) with honey (15) butter Milk (100)
Supper	Plain souffle, egg (100) milk (50) Carrot (50) and peas (25) salad on lettuce (25) Orange ice (100) Bread—whole wheat (25) Milk (100)	Scrambled eggs (75) Tomato soup (60) with peas (25) Apple (50) and lettuce (25) salad Slice banana (75) with milk (50) Milk (50) Bread—whole wheat (25)

* Figures in parenthesis indicate amounts in grams for twelve-year-old child. Foods were suitably seasoned from day's allotment of butter, salt and sugar.

customed in the experimental diets. One of the subjects, little Billy, on seeing one of the familiar articles of diet reached for it with zest, exclaiming, "good old carrot sticks."

Provision of Essential Dietary Components

It is essential that the nutritive constituents of food be furnished in ample amounts and in proper proportions to satisfy the

TABLE 18

VARIETY IN USE OF FOODS

Apple: Cored and quartered or carved for Jack-O-Lantern; in salads, with cheese, peanut butter, carrot, banana, jelled orange juice; in fruit cup; sliced in sandwiches. Cooked in apple sauce; fried apple rings; apple Betty, with graham crackers; apple souffle; baked, plain or stuffed with banana, peanut butter or ground beef.
Banana: Sliced, with corn flakes, milk or orange juice; in salads, with peanut butter, cheese, apple, and jelled orange juice; fruit cup or jelled orange juice; mashed, plain or with peanut butter, for sandwich filling, salad dressing, cake frosting or ice cream. Baked, plain or with honey, peanut butter or apple; banana pudding with milk and egg; Betty with graham crackers.
Orange juice: Plain beverage or orangeade; in fruit cup with apple and banana; jelled with gelatin; frozen as orange ice; in pudding with milk and egg.
Tomato juice: Plain beverage; jelled for aspic salads, with cheese, peas, carrots or hard boiled egg. Cooked in soup, plain or with milk, peanut butter, peas, cabbage or cheese; in stew with meat, vegetables or bread.
Bread: Buttered, plain; sandwiches; toast; bread and milk or milk toast; toast for baked or creamed eggs; cooked with tomato juice, as dressing, with souffles or puddings.
Corn flakes: With milk, plain or with sliced banana, apple sauce or heated with honey. Cooked as "flour" medium in pudding and cake; candied with sugar-water syrup.
Graham crackers: Buttered plain or spread with honey, peanut butter, apple slices, mashed banana; with milk. Cooked in apple or banana Betty, meat loaf, and cake.
Potato: Boiled, fried, browned, mashed (plain or as timbals for peas, baked egg), escalloped; baked, plain or with meat in hash, souffle, soup, stew.
Cabbage: Shredded, in salads. Creamed, plain or with other vegetables; stew, borsch with beef and tomato juice; souffle.
Carrots: In salads as sticks, diced or shredded; with cheese, peanut butter or in jelled orange juice. Buttered or creamed, plain or with peas; meat or vegetable loaf; souffle.
Peas: In salads, alone or with cheese, carrots; aspic salad. Buttered or creamed, plain or with carrots, potatoes or eggs; soups.
Beef, ground: Fried in pattie and served alone or in sandwich; hot beef sandwich or on mashed potato. Stew with tomato juice and vegetables; hash with potatoes; borsch with cabbage; baked with potato; meat loaf with graham crackers and egg.
Cheese: Plain, in sandwiches or on graham crackers; toasted sandwiches or melted on toast; salads with peas, apples, carrots, in aspic salad. With escalloped potatoes, cabbage, creamed or baked eggs; with potato soup; souffle.
Egg: Egg nog or ice cream. Scrambled, baked, fried, hard-boiled (plain in salad or creamed), souffles, puddings, custards, cake, meat or vegetable loaf.
Peanut butter: Sandwiches or on graham crackers, plain or with banana, apple, honey; salads with apple, banana, shredded carrots, or as dressing. Baked with apple or banana; tomato soup; frosting with banana for cake.
Honey: Sandwiches or on graham crackers, plain or combined with banana or peanut butter; honey-butter for toast; sweetening fruit cup, gelatin, orange juice, apple sauce, corn flakes, puddings.
Gelatin: Jelled aspic salads; meat loaf or plain pattie; stew and soup.

energy, maintenance and growth needs of children. The energy requirement fluctuates[38] according to the metabolic state and activity of the body. Carbohydrates and fats are used continuously to meet these needs but, if needed, more of the amino acids derived from the protein moiety may be converted into glucose and used for the additional energy required. During such a metabolic demand these amino acids are no longer available for the synthesis of new tissue protein. Thus, ample provision of carbohydrate and fats in the diets of growing children tends to "spare" the protein moiety, and increase its effectiveness in meeting the body requirements specifically related to amino acids.

The many possible causes of dental caries offer fertile fields of investigation. There are studies which seem to indicate that destruction of enamel and dentin in dental caries is caused by the action of bacteria which have the ability to ferment sugars and convert them into acids, the acids attacking those surfaces of the teeth with which they come in contact. Dental investigations including chemical and bacteriological studies of the saliva, have shown a high relationship between sugar in the diet and the occurrence and degree of dental caries activity, associated with the presence of lactobacilli and acidity of the saliva. Although all causes of dental decay are not known, high sugar consumption tends to furnish a more favorable medium for lactobacillus activity in the individual who is susceptible to dental caries.[40a] High sugar intake may lower resistance in the immune individual. Feeding purified, processed foods such as sugar to satisfy energy requirements is not prudent when natural foods may be given which meet energy requirements and at the same time convey certain vitamins and other nutriments which aid in carbohydrate and fat metabolism and more satisfactory energy utilization in the body.

If a dietary is adequate in quantity and quality of all nutriments the healthy child will not have an abnormal craving for sweets, especially sugar. This fact is substantiated by our observations of the free choice of sugar by healthy children, over the past ten years. As a diet is carefully adjusted to meet the particular needs of an individual child, the healthy child voluntarily reduces his sugar consumption as his bodily requirements are more perfectly met (Figure 36) through a better balanced diet of natural foods. We have permitted freedom in sugar intake among our experimental subjects and have recorded the quantity of sugar voluntarily consumed daily by each individual; we have used this as one of the criteria for adequacy of the food intake. In 519 observations over 2595 days, 29 children voluntarily chose about ten grams (average) of refined sugar daily, which affirms the adequacy of their diets.

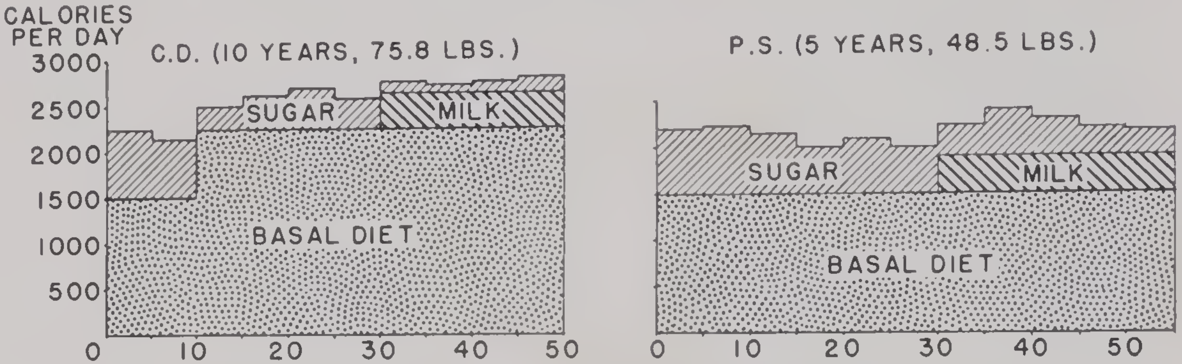

FIGURE 36. Voluntary decrease in sugar consumption by two children following increased adequacy of diet.

The diets of the children in these studies contained the average quantities of protein shown in Table 19. These figures agree with the general practice of providing 2.2 to 3.0 gm. of protein per kilogram of body weight per day in diets in which the total calories are ample. On the average, protein furnished approximately 15 per cent of the total calories, which is in accord with the 10 to 15 per cent considered liberal for childhood by Rose.[281]

TABLE 19

AVERAGE DAILY PROTEIN INTAKE OF 29 CHILDREN DURING 2965 EXPERIMENTAL DAYS*

Age	Protein intake	
yr.	gm.	gm./kg.†
4	59.9	3.26
5	60.9	3.25
6	63.6	2.92
8	72.2	2.77
9	81.6	2.86
10	81.5	2.46
11	82.9	2.32
12	82.9	1.98

* In Table 20, page 87, are shown the distribution of the subjects among the age groups; the distribution of the five-day balance periods among the subjects; and, the average body weights and recumbent lengths of the subjects in each age group during the balance periods of study.

† Per kilogram of body weight.

In growth it not only is necessary to have sufficient protein in the diet but it must be of proper composition for construction of new protoplasm. Furthermore, some proteins have a higher biological value than others, that is, they are more readily and completely digested and utilized in the body. A liberal quantity of protein in the diet, chosen from a variety of sources is indicated during growth, to insure an adequate supply of the essential amino acids.

Amino Acids: Until attention was directed toward possible differences in nutritive quality, emphasis had always been placed upon the quantity of protein consumed. The importance of adequate allowances of proteins containing the amino acids which cannot be synthesized in the body and which are indispensable for growth and maintenance has been stressed in recent years. The early studies contributing to this knowledge were initiated by Osborne and Mendel.[256] Other investigations were inaugurated by Hart and Steenbock, at the University of Wisconsin; by McCollum and Simmonds,[213] at The Johns Hopkins University; and, more recently, by Rose and Mitchell, both at the University of Illinois. These observations have been extended by the amino acid analyses of Vickery[359] and coworkers, at the Connecticut Agricultural Experiment Station in New Haven. The results of these and other equally significant investigations have led to the selection of dietary proteins on the basis of meeting daily requirements for the essential amino acids.

The nutritive superiority of one protein over another appears to be connected with the distribution of the nutritionally essential amino acids. It is known that no two proteins are exactly alike in composition and that some proteins are notably lacking in certain essential amino acids. A food protein carrying only a trace, or none, of one or more of the essential amino acids may be supplemented by another incomplete food protein containing the missing essentials, thereby furnishing an adequate amino acid mixture. The proteins of milk, cheese, eggs and meat have been used to furnish all the known essential amino acids for growth and to supplement the incomplete proteins of cereals and vegetables. It has been shown that 30 to 50 per cent of the maintenance nitrogen requirement in the adult rat can be satisfied by a variety of incomplete amino acid mixtures, but that the remaining 50 per cent, or more, of the nitrogen allowance must be contained in specific amino acid structures.[41] In compounding a diet, cereals, fruits and vegetables should be supplemented with generous proportions of meat, milk, eggs and cheese. Such a dietary, if given in ample quantity, will meet amino acid requirements of the body.

TABLE 20

AVERAGE LENGTHS AND WEIGHTS OF 29 CHILDREN WHO WERE SUBJECTS OF METABOLIC BALANCE STUDIES DURING 593 BALANCE PERIODS

Age group	Subjects	Five-day balances determined	Average body weight	Average recumbent length
year	number	number	kg.	cm.
4	5	59	18.44	108.8
5	5	158	18.74	111.0
6	6	186	21.83	119.0
8	6	114	26.16	129.7
9	2	22	28.58	136.0
10	3	32	33.21	139.8
11	1	11	35.75	138.3
12	1	11	41.84	159.1

Only recently have preliminary analyses of the amino acid content of the protein mixtures in human foods become available; in these the nitrogen partition among the amino acids is only partially known. Satisfactory methods for quantitating the amino acids have evolved slowly, because of the complexity of the problems encountered in analytical procedures. A method developed with pure proteins may fail when applied to natural foods. Interfering substances and destruction of certain amino acids during the processes of preparing the native foods for analysis have been barriers to successful determination and fuller knowledge of the amino acid composition of our common food supply. Some of the data available represent only approximate compositions but progress is being made towards more exact knowledge of the partition of the amino acids in foods.

A tentative estimation of the amino acid composition of diets consumed during childhood, using the scant data at present available,* has seemed to us worth while, although all the limitations mentioned above must be recognized in any interpretation or application of the values. The individual foods in the diets given to the children during study of nutrition and chemical growth were analyzed for their nitrogen contents. Those foods with established values for some of their constituent amino acids are given separately in Table 21. These amino acids accounted for 5.4 gm. of the average daily intake of nitrogen (13.0 gm.). The foods for which partial amino acid analyses are available contained 11.6 gm. of nitrogen, 90 per cent of the daily intake. The remainder of the daily nitrogen intake was contained in the foods included in the diet for which no amino acid values are available.

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* The data on the amino acid composition of foods were selected from various sources in the literature; from data collected in this laboratory; and, some were generously made available to us prior to publication through the kindness of Dr. Richard Block.

TABLE 21

NITROGEN CONTENT OF INDIVIDUAL FOODS IN DAILY DIET OF 12 YEAR OLD CHILD*

Foods for which partial amino acid analyses are available	Intake		Foods for which amino acid analyses are not available	Intake	
	Food	Nitrogen		Food	Nitrogen
	gm.	gm.		gm.	gm.
Beef, lean	100	3.14	Apple	100	0.04
Bread, white	50	0.78	Banana	150	0.25
Bread, whole wheat	50	0.89	Cabbage	25	0.05
Corn flakes	30	0.34	Carrot	50	0.05
Cheese, American	20	0.61	Lettuce	25	0.03
Egg, whole	100	1.70	Orange juice	100	0.17
Graham cracker	36	0.40	Peas	25	0.27
Gelatin	3	0.49	Potato	120	0.42
Milk	500	2.54	Tomato juice	60	0.08
Peanut butter	16	0.74			
Total		11.63			1.36

* In deriving the data given in Table 22, from these values, the beef nitrogen was distributed according to amino acid analyses of beef muscle protein; the nitrogen contents of white bread, whole wheat bread and graham crackers were added and distributed according to analyses of wheat gluten; the corn flake nitrogen was distributed according to analyses of corn gluten; the nitrogen contents of milk and cheese were added and distributed according to analyses of whole cow's milk proteins; egg was calculated from whole egg protein analyses, and peanut butter nitrogen was distributed from analyses of arachin, which accounts for at least 80 per cent of the nitrogen in the peanut.

It is known that some nitrogen in the foods is not in the form of amino acids, although it is not likely that the nonprotein nitrogen of any of the foods exceeds 10 per cent of the total nitrogen. Since partial amino acid distribution values were not available for about 10 per cent of the dietary nitrogen, it is probable that the approximation given in Table 22 is near the level of the actual intake. The table expresses the daily dietary in terms of the total amounts of 12 amino acids obtained from animal and vegetable proteins. The animal proteins of the diet used in our studies furnished the greater amounts of all the essential amino acids, owing to the fact that animal sources were used to provide the larger amounts of protein.

At the present time it is recognized that lysine, tryptophane, histidine, phenylalanine, leucine, isoleucine, threonine, methionine, and valine are essential for the growth of rats consuming an otherwise adequate diet, for the withdrawal of any one of these amino acids from the diet results in cessation of growth. Growth will occur at a subnormal rate in the absence of arginine but it must be present for normal growth to take place.[283] Holt and his coworkers have recently demonstrated that tryptophane and lysine are among the amino acids essential for maintenance of nitrogen equilibrium in adult man.[1,145] Cystine is not essential in the diet of the rat, to produce growth, but its presence reduces the methionine requirement. This fact illustrates the synergistic relationship which exists between amino acids which are not essential and those which are indispensable for growth. Studies of the essential nature of the amino acids, in which mixtures of amino acids have served as the sole source of nitrogen, have recently been extended by Rose and Rice,[284] who procured, with

TABLE 22

ESTIMATED AMINO ACID CONTENT OF THE DIET OF A 12 YEAR OLD CHILD

	AMINO ACID INTAKE		
	Animal proteins	Vegetable proteins	Total
	gm.	gm.	gm.
Cystine	0.63	0.31	0.94
Methionine	1.85	0.50	2.35
Arginine	3.17	1.09	4.26
Histidine	1.06	0.35	1.41
Lysine	3.72	0.44	4.16
Tyrosine	2.58	0.88	3.46
Tryptophane	0.70	0.13	0.83
Phenylalanine	2.82	0.96	3.78
Threonine	2.31	0.56	2.87
Valine	2.23	0.60	2.83
Leucine	6.80	1.85	8.65
Isoleucine	2.22	0.55	2.77
Total	30.09	8.22	38.31
Nitrogen intake from foods of known amino acid content (Table 21)	8.48	3.15	11.63
Nitrogen intake accounted for by 12 amino acids	4.30	1.17	5.47

dogs, results identical to those obtained with rats. These authors conclude that since two widely different mammalian species require the same components of the protein molecule for health, there is a high probability that man may have a similar requirement.

In the light of the present knowledge, gained in animal investigations,[282] it is of interest to examine the dietary given to children in accordance with the quantitative needs for each of the indispensable amino acids. Rose[284] proposes that "mixtures of purified amino acids, compounded in accordance with the quantitative needs of the cells for each component may prove to be the most efficient type of nitrogen ever devised for the uses of the animal organism." This proposal is already being put to a test in the clinic.[75,93,313]

Carbohydrate and Fat

The average intakes of carbohydrate and fat provided by the diets given in the studies of nutrition and chemical growth are shown in Table 23. Consumption of 11.4 to 6.2 gm. of carbohydrate per kilogram of body weight per day in childhood (4 to 12 years) includes the range of 8 to 10 gm. which is considered adequate for children under usual conditions. On the average, 45 per cent of the determined gross calories was in the form of carbohydrate, a figure consistent with the 40 to 60 per cent which is considered the optimal range.

TABLE 23
AVERAGE DAILY CARBOHYDRATE AND FAT INTAKES OF 18 CHILDREN DURING 2510 EXPERIMENTAL DAYS*

Age group	Carbohydrate		Fat	
yr.	gm.	gm./kg.†	gm.	gm./kg.†
4	205	11.4	71.2	3.95
5	191	10.3	67.4	3.60
6	206	9.5	74.1	3.39
8	240	9.1	91.3	3.47
9	249	8.7	82.8	2.90
10	260	8.0	112.8	3.45
11	261	7.3	112.8	3.15
12	261	6.2	112.8	2.69

* See Table 24, page 92 for body weights and lengths of subjects, distribution among age groups and number of balance periods for each group.

† Per kilogram of body weight.

Carbohydrate not only is the most easily available and economical source of energy but also its metabolism is a requisite for the metabolism of fats and proteins. Fat is the richest source of energy to the body. Weight for weight it provides more than twice the energy released by protein or carbohydrate. The chemical mechanism by which the energy of fat becomes available for muscular work has been the subject of many investigations but is not yet completely solved.[24] Recent authoritative work[326] indicates that much of the fat used passes through the ketone body stage and is completely burned; that ketone bodies appear only when an excess of fat is being burned; and that carbohydrate, by preventing excess use of fat, prohibits excessive ketone body formation and waste. Fat, like carbohydrate, exerts a "sparing" action on the protein in the processes of metabolism. About 38 per cent of the gross calories of the diets came from fat, averaging, for all the children, 3.5 gm. per kilogram of body weight. The latter figure compares favorably with the findings of Holt,[143] that healthy children receive from 2 to 4 grams of fat per kilogram of body weight per day.

Ketogenic-Antiketogenic Ratios: The oxidation of carbohydrate is independent of the presence of fat in the diet but the presence of carbohydrate aids in fat utilization. About 75 per cent of the energy contained in protein can be released in the animal body. Newburgh and his students[248] have summarized the practical dietetic problem. It is necessary to know how much fat is to be oxidized in relation to the available supply of glucose. The organism is not at all dependent upon glucose in carrying out the first step; that is, in splitting the neutral fat into fatty acid and glycerol. The limitation occurs in the oxidation of the fatty acid. When sufficient glucose is at hand, the fatty acid is completely oxidized to carbon dioxide and water, but a shortage of glucose results in incomplete oxidation and ketone bodies, beta-oxybutyric acid, diacetic acid and acetone enter the blood. Ninety per cent of the neutral fat that is brought into play must be dealt with as fatty acid. It has been shown that the amino acids leucine, tyrosine and phenylalanine all may form some ketogenic substances during metabolism. In order that the fatty acids be oxidized and accumulation of ketone bodies in the blood and urine avoided, the keto-antiketogenic ratio* of the

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* The keto-antiketogenic ratio is the relation between the total amount of potential ketogenic substance in the diet and the total amount of antiketogenic substance and is calculated as:

$$\frac{\text{Fatty Acid}}{\text{Glucose}} = \frac{0.44 \text{ Protein} + 0.9 \text{ Fat}}{\text{Carbohydrate} + 0.58 \text{ Protein} + 0.1 \text{ Fat}}$$

dietary assumes importance in health and disease [DuBois[68]]. This is especially true in children's dietaries, since their bodies are less efficient than those of adults in oxidizing fatty acids to completeness, e.g., carbon dioxide and water. It is not unusual to find children in ketosis after engorgement of a high fat dietary. Glucose or its derivatives make up the antiketogenic substances. Assuming that protein furnishes 58 per cent of its weight in glucose, that carbohydrate furnishes 100 per cent and that fat (the glycerol fraction) furnishes 10 per cent of its weight in glucose, the antiketogenic value of the diet may be calculated.

TABLE 24

AVERAGE LENGTHS AND WEIGHTS OF 18 CHILDREN WHO WERE SUBJECTS OF METABOLIC BALANCE STUDY DURING 2510 EXPERIMENTAL DAYS

Age group	Subjects	FIVE-DAY BALANCES DETERMINED		Average body weight	Average recumbent length
		Fat	Energy		
year	number	number	number	kg.	cm.
4	1	43	42	17.99	108.6
5	3	125	119	18.66	111.2
6	4	170	167	21.77	119.3
8	4	98	94	26.33	130.0
9	2	22	22	28.58	136.0
10	2	22	22	32.72	142.3
11	1	11	11	35.75	138.3
12	1	11	11	41.84	159.1

The average daily dietary of 18 children of this study, containing 68 grams of protein, 79 grams of fat and 215 grams of carbohydrate, had a keto-antiketogenic ratio of 0.38. Although present methods do not permit exact estimations of the various substances formed from fat and protein in metabolism, if the total amount of fatty acids divided by the total amount of glucose falls below 1.5 there will be no sign of ketonuria. According to the keto-antiketogenic ratio of 0.38 in the children's dietaries, there was an abundance of glucose over the fatty acids. Newburgh[248] states that one may expect acidosis to begin when the amount (by weight) of ketogenic substance (fatty acid) is four times that of the antiketogenic substances.

Minerals

Minerals are used in varying proportions in the body both as building materials and as body regulators.[79] Calcium and phosphorus in definite proportions are used for the formation of bones and teeth; phosphorus, potassium, sulfur and chloride are used to construct soft tissues such as muscle, nerves and glands; iron, copper, calcium, phosphorus, sodium, potassium and chlorine, for blood and its component parts; iodine for thyroid gland development and chlorine for gastric secretion; zinc for carboxylase activity. Sodium, potassium, magnesium and chlorine are essential to the movement of water into and out of the cells and the exchange of body fluids in general; maintenance of the proper balance between the positive (calcium, magnesium, sodium and potassium) and negative (phosphorus, chlorine and sulfur) minerals is essential to electroneutrality in the body. Iron, zinc, iodine, magnesium, manganese and possibly other elements are needed for carrying on oxidation processes; calcium, sodium, potassium and magnesium in the contractibility of muscles; calcium in the irritability of nerves and the clotting of blood.

TABLE 25

AVERAGE DAILY INTAKE OF POSITIVE AND NEGATIVE MINERALS BY 29 CHILDREN DURING 2965 EXPERIMENTAL DAYS*

Age group	Positive minerals (mg.)				Total positive minerals	Negative minerals (mg.)			Total negative minerals	Alkaline-ash value†	
year	Ca	Mg	Na	K	meq.	P	Cl	S	meq.	meq.	meq./kg.
4	829	290	2128	2565	223.4	1141	3360	684	203.7	19.7	1.09
5	867	284	2101	2574	223.9	1152	3324	679	203.1	20.8	1.11
6	823	287	2162	2629	226.0	1130	3437	692	205.8	20.2	0.92
8	1130	316	2471	2955	265.5	1416	3826	808	240.6	24.9	0.95
9	942	291	2997	3252	284.5	1464	4339	973	268.1	16.4	0.57
10	1197	356	3009	3560	311.0	1714	4435	1027	288.8	22.2	0.67
11	947	304	3013	3487	292.6	1501	4359	999	272.5	20.1	0.56
12	947	304	3013	3487	292.6	1501	4359	1000	272.5	20.1	0.48

* See Table 20, page 87 for body weights and lengths of subjects, distribution among age groups and number of balance periods for each group.

† Excess of positive minerals over negative minerals.

In addition to quantitative intake of the individual positive and negative minerals, the acid- or alkaline-ash value of a combination of foods must be considered in planning diets. The average intakes of positive and negative mineral elements from the diets ingested by the children are summarized in Table 25. The intakes provided a liberal supply of minerals, both for current growth requirements and the maintenance of all body processes. Moreover, the diets contained an excess of positive minerals, a requirement for growth. The alkaline-ash values ranged from 0.48 to 1.11 milliequivalents per kilogram per day. These amounts of positive minerals in excess of negative minerals are known to favor utilization of calcium and phosphorus.

Heavy Metals

The amounts of zinc, copper, manganese and nickel ingested in a dietary composed with common foods are surprisingly large. The diet given a 12 year old boy for 55 consecutive days supplied an average of 16.3 mg. of zinc per day, an amount approximately 34 per cent greater than the average daily intake of iron (12.2 mg.). The same diet provided, daily, averages of 5.2 mg. of copper; 2.2 mg. of manganese and 1.2 mg. of nickel.

Vitamins

The vitamins are necessary as regulators and, in adequate quantities and proper proportions to one another, aid in effective utilization of the minerals, carbohydrates, protein and fats in growth and in maintenance of health. Each vitamin has characteristic properties and specific physiological functions in the body. Frequent bioassays were made of the vitamin D content of the milks and titrations made for the vitamin C content of the orange juice, because these foods provided appreciable quantities of these accessory food factors. We have estimated the vitamin intakes of the experimental subjects, insofar as values for the individual foods in the diets are available.

The values assigned to the foods in the diet of a 12 year old child are shown in Table 26. The average daily vitamin intake provided by the diets used in our studies, for vitamin A, thiamin (B_1), ascorbic acid (C), niacin (nicotinic acid), and riboflavin (B_2) (Table 27), were above the allowances recommended by the Committee on Food and Nutrition, National Research Council.[52]

Recent evidence shows that increasing the caloric intake of animals fed too little thiamin (B_1) increases their liability to deficiency disease.[56] Moreover, increased carbohydrate consumption hastens the onset of polyneuritis, fat has a retarding influence and protein has a less pronounced effect in either direction.[390] The close relationship observed between calorie and thiamin intakes enhances interest in thiamin intake in relation to the total calories,[56] carbohydrate calories, and the non-fat-calories consumed by children. Since Williams and Spies[390] have shown that the vitamin-calorie ratio required for a given species appears to be independent of the weight of the individual, within the limits of experimental error, no attempt has been made to evaluate the diets in relation to increasing body weight. The exact requirement of man is not known and it varies among indi-

TABLE 26

ESTIMATED DAILY VITAMIN INTAKE OF A 12 YEAR OLD BOY*

Food	Amount		Vitamin A	Thiamin	Riboflavin	Niacin (Nicotinic acid)	Ascorbic acid	Pantothenic acid	Pyridoxin (Anti-acrodynic potency)
	Wet	Dry							
	gms.	gms.	I.U.	μg.	μg.	mg.	mg.	μg.	Schneider units
Apple	100	11.5	75	45	73	0.05	10.8	4.4§	25
Banana	150	35.1	450	72	126	6.32§	16.5	105	99
Lean beef	100	31.2	50†	120†	225†	3.80	0	1000	39§
White bread	50	32.6	—	22	—	0.40	0	—	—
Whole wheat bread	50	34.4	—	120†	52†	1.50	0	—	200
Cabbage	25	1.6	22†	18†	18†	0.08	8.8†	—	—
Carrot	50	5.6	1050†	30†	29†	0.25	2.4	100	—
American cheese	20	11.8	400	8	150	—	—	—	50
Whole egg	100	21.4	1000	150	330	0.10	0	2700	178£
Lettuce (head)	25	1.3	25	22	12	—	1.2	—	6
Milk	500	63.45	550†	254†	1042†	0.36#	6.2†	1400	200
Orange juice	100	12.6	198	90	15	—	41.2	70+	16+
Peanut butter	16	14.6	—	144‡	10	2.88‡	0	848‡	266‡
Potato	120	23.5	49†	148†	56†	2.40	4.1†	780	48
Tomato juice	60	3.5	600†	55†	37†	—	14.4†	60¶	15¶
Graham cracker	36	31.7	—	—	—	—	—	—	—
Salt	2	2	—	—	—	—	—	—	—
Butter	60	—	1440	63	0	—	0	—	120
Corn flakes	30	28.4	—	—	—	0.52	—	—	—
Peas	25	5.5	250	105	36	0.31	6.5	4§	—
Honey	15	12.4	—	—	—	—	—	—	—
Gelatin	3	2.7	—	—	—	—	—	—	—
Sugar	22.3	22.3	—	—	—	—	—	—	—
Total	1659.3	412.15	6159	1466	2211	19	112	7071	1262
Standard†			4500	1200	1800	12	75		

* Except as footnoted, the following standards were used: for vitamin A, thiamin, riboflavin and ascorbic acid, those given by Eddy and Dalldorf;[3] for nicotinic acid, those given by Elvehjem, in a personal communication; for pantothenic acid, those published by Jukes;[169] for pyridoxin content, those given by Schneider.[293] When ranges were given as standards, the midpoint was used. International Units of thiamin and ascorbic acid were converted by the factors given by Munsell:[216] Number I.U. ×3 = micrograms of thiamin. Number I.U. ×0.05 = milligrams ascorbic acid.

† Committee on Food and Nutrition, National Research Council, May 1941.[52]

‡ Peanuts or peanut meal.

§ On dry basis.

# Values of Noll and Jensen.[252]

+ Orange.

¶ Tomatoes.

£ Egg yolk was estimated as one-third of the raw weight of the total egg.[234]

viduals and for the same individual at different times depending upon the existing physiological state, and under varying environmental temperatures.[237] Furthermore, in the estimation of the quantity of thiamin, the wide discrepancies in the results obtained by expert technicians in different scientific laboratories, working on the same product, defy precise assignment of quantitative values to any one diet. Then, too, the vitamin content of the same food varies, depending upon climate and the soil in which it is grown, upon the way it is handled, the length and manner of aging, the way the food is cooked and the temperatures and environment of the cooking and serving of the food.

TABLE 27

COMPARISON OF RECOMMENDED DAILY ALLOWANCES OF VITAMINS* AND VITAMIN INTAKES OF EXPERIMENTAL SUBJECTS

	RECOMMENDED*			GIVEN†		
	4–6 years	7–9 years	10–12 years	5 years	8 years	11 years
Vitamin A (I.U.)	2500	3500	4500	3752	5415	6159
Thiamin (mg.)	0.8	1.0	1.2	1.0	1.4	1.5
Ascorbic acid (mg.)	50	60	75	75	110	112
Riboflavin (mg.)	1.2	1.5	1.8	1.6	2.2	2.2
Niacin (mg.)‡	8	10	12	15	18	19
Pantothenic acid (mg.)				5.0	6.7	7.1
Pyridoxin (Schneider units)				925	1178	1262

* Allowances recommended by the Committee on Food and Nutrition, National Research Council,[52] as a tentative goal toward which to aim in planning practical dietaries; can be met by a good diet of natural foods. Such a diet will also provide other vitamins, the requirements for which are less well-known. Allowances are based on needs for the middle year in each group, and for moderate activity.

† Calculated for the diets given children 5, 8 and 11 years old from the contents of the foods for which analyses were found in the literature (see Table 26). The children received supplementary amounts of vitamin D from irradiated milk and spent several hours each day playing in the sun.

‡ Nicotinic acid.

Although the total amount of energy consumed was increased with age, the percentage of the gross caloric intake represented by carbohydrate decreased from 46 to 43; the percentage in the form of fat increased from 37 to 43, with children of increased ages, from 4 to 12 years. If fat does have a sparing action upon thiamin, then the older children not only had an advantage in receiving a relatively larger quantity of this vitamin but also the caloric intake was distributed to give the older children an advantage.

The average daily thiamin intakes were at least 1000 micrograms for the four year old children and 1400 micrograms for the older subjects. These amounts are well above the amounts estimated as adequate for adults ingesting diets of much higher caloric value. The ratios of thiamin intake* to the net Calories of the diets (physiologic fuel value determined by subtracting heat of combusion of urine and feces from that of food intake) showed only insignificant variations for the diets fed to the different children. For the diet given to a 12 year old child (Table 26) the ratio of thiamin:total net Calories was 0.6; of thiamin:carbohydrate Calories, 1.4; of thiamin:fat Calories, 1.4; and of thiamin:non-fat Calories, 1.2. According to Williams and Spies' estimate, the thiamin to non-fat caloric ratio necessary to protect against beriberi is considered very close to 0.3 in comparison with 1.2 in this study.

Water

Water is required as a body builder and body regulator. It is an essential constituent of every kind of tissue, serves as a solvent and plays a dominant role in the formation of all the body fluids and secretions as well as the excretions. In addition, it assists in the regulation of body temperature. The total daily water consumption, inclusive of the water associated with foods, ranged from 1685 to 1745 ml. for children 8 to 12 years of age, 449 to 568 ml. of which was drinking water.

Complex Carbohydrates

Some bulk or indigestible vegetable fiber is necessary in the diet, for the maintenance of desirable intestinal tone and stimulation of intestinal muscles, to assist in producing satisfactory gastrointestinal activity and regulate bowel elimination. Raw fruits and vegetables, and whole wheat cereals serve as the main source of this bulk.[208] Vegetable fiber is made up of cellulose, hemicellulose and lignin and is sometimes referred to as unavailable carbohydrate because the body contains no known enzymes to digest it, although it may be broken down in the alimentary tract through bacterial action. The average complex carbohydrate contents of the diets are recorded in Table 28. The intake of total fiber was greater than the physiologic roughage minimum of 90 to 100 mg. per kilogram of body weight that Cowgill and Anderson[51] considered necessary for satisfactory elimination in healthy, adult men. There was no indication that the quantity given was not entirely adequate to satisfy the physiological needs of the children. During all of the studies not one laxative was given.

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* All ratios are given in micrograms of thiamin to net Calories.

TABLE 28

AVERAGE DAILY COMPLEX CARBOHYDRATE INTAKE OF 18 CHILDREN DURING 2455 EXPERIMENTAL DAYS*

Age group	Lignin	Cellulose	Hemicellulose	Total	
year	gm.	gm.	gm.	gm.	gm./kg.
4	1.51	2.25	2.21	5.97	0.33
5	1.42	2.23	2.20	5.85	0.31
6	1.48	2.39	2.45	6.32	0.29
8	1.36	2.41	2.53	6.30	0.24
9	1.25	2.74	2.80	6.79	0.24
10	1.26	2.88	2.87	7.01	0.21
11	1.26	2.88	2.87	7.01	0.20
12	1.26	2.88	2.87	7.01	0.17

* See Table 24, page 92 for body weights and lengths of subjects, distribution among age groups and number of balance periods for each group.

CHAPTER V

SOME PHYSIOLOGICAL ASPECTS OF DIGESTION AND EXCRETION

MENDEL[235] pointed out several years ago that "one should recognize in the study of alimentation something more than the mere consideration of the solvent action which the digestive secretions can exert, . . . but [it] is the consideration of the co-operative working of many functions—an ordered march of events." In any metabolic study it is important to consider the characteristic biological pattern for the different segments of the alimentary canal, the interdependence of cooperation between various physiologic tissues in the biochemical changes which proceed within the tract, and the absorption and utilization that take place and the resultant general fitness and response of the body as a whole. Indeed, Mendel[235] emphasized that "the relationship which exists between the functions of the digestive tract and that ensemble of biologic processes termed nutrition is both manifold and far-reaching." The primary functions of digestion are indispensable in the preparation of food for passage through the alimentary canal and subsequent assimilation. To obtain an adequate evaluation of gastrointestinal activity in the growth and nutrition of normal children it is necessary to train the human subjects in metabolic technique (p. 47), thus enabling complete cooperation and reducing to a minimum emotional disturbances such as fear, anxiety, and anger.[45,46,348]

Digestion and absorption of food depend upon mastication and mixing with salivary secretions, the type and condition of the food in the stomach, and subsequently in the intestinal tract, and the length of exposure to the various digestive and absorptive processes. Many factors are interrelated in the activity of the alimentary canal; the size of the meal ingested,[348,354] its consistency and contents;[279] the nutritional state of the individual; and, the emotional environmental conditions to which the individual is subjected at the time.[348] The mastication of food is an important factor in gastrointestinal activity, for food finely divided through attrition is more completely exposed to alimentary secretions and causes less mechanical irritation of the lining of the tract. Dental and mouth conditions may sometimes be a deleterious factor inhibiting proper mastication. It has been demonstrated that gastric tone and the force of gastric peristalsis may be changed by different stimuli, applied in sufficient strength to the small and large intestines. Little absorption occurs from the stomach, although the organ carries out important digestive functions by its mechanical churning action and its enzyme system. Gastric juice, which is strongly acid and carries the proteolytic enzyme, pepsin, exerts a hydrolizing action on proteins by breaking them down to proteoses and peptones. Some digestion of lipid may also occur.

Among the conditions within the intestine that affect gastric motor activity are mechanical distension, chemical irritation, the action of hypertonic or hypotonic solutions, and the presence of products of protein and starch digestion. Fat[272] and carbohydrate[167,273] inhibit gastric secretion and motility through a humoral mechanism whose active principle has been given the name of enterogastrone. This mechanism which affects gastric secretion and motility no doubt exerts a strong influence in providing suitable conditions for digestion of the various foodstuffs.

The physiologically beneficial effects of cellulose[54,55,280] and, more recently, the recognition of the component parts of "crude fiber" in satisfactory elimination has gained dietetic consideration[92,130,254,387] although their exact physical and physiological activities within the human body are not fully understood. Some individuals have a superior ability to decompose fibrous food substances in transit through the alimentary canal, because of a particular bacterial flora or other factors. In such individuals, insufficient quantities of roughage may remain to stimulate elimination. Some foods contain pectin, which seems to have unique physiological value in stimulating more wholesome utilization and passage of intestinal contents.[231] Other foods contribute to satisfactory buffer action[122] and effect a change in the bacterial flora of the feces,[236] all of which may have an effect upon elimination and gastrointestinal activity, and, consequently upon the utilization of food elements.[160,229] Studies indicate, however, that the unavailable carbohydrates may stimulate protein retention and that the character of the fiber (i.e., the proportion of lignin to cellulose and hemicellulose) may have physiological importance in the diet.[305] The decomposition or fermentation of the crude fiber in the digestive tract of man is affected by the age and makeup of the plant cell membrane, the proportion of the component unavailable carbohydrates, and by the dietary mixture as a whole. A physiologic roughage minimum of 90 to 100 mg. per kilogram of body weight has been considered necessary for satisfactory elimination in man.[54]

Gastrointestinal Activity

Length of exposure to the various digestive and absorptive processes which occur in the gastrointestinal tract exerts a major influence upon the efficiency with which nutriments are assimilated. During ingestion of a meal the stomach is filled with masticated food, carrying the salivary digestive enzymes. Salivary digestion inside the food bolus and gastric digestion at the outer portions proceed simultaneously. Some of the ingesta may pass rapidly through the stomach and egress into the intestine with scarcely any opportunity for exposure to pepsin and other gastric secretions, while the last portion of the meal may be retained in the stomach until salivary and gastric digestion are quite complete. Little absorption occurs from the stomach, but the ingesta undergoes the most profound digestive changes in the small intestine and it is here that most of the products of digestion are absorbed.

The intestine receives the contents of the stomach much more slowly than the stomach received the original food (Figure 37). Each portion, as it is ejaculated from the stomach, becomes subject to the propulsive, peristaltic action of the intestine. Intestinal digestion begins in the duodenum, about 8 to 10 cm. from the pylorus, where the secretions from the bile and pancreatic ducts empty and combine their action with that of the *succus entericus*, thus introducing rapid and powerful proteolytic, amylolytic and lipolytic enzymes whose activities proceed simultaneously, along with organic and inorganic buffer systems which maintain a favorable *pH* for activity of these enzymes. Absorption of digestion products favors completion of enzyme activity. Digestion and absorption *may* continue in the large intestine, where excreted material is added to the residue from digestion.

It is evident that the fundamental process of growth, conversion of food into tissue, primarily is dependent upon adequate digestive functioning. It is not inconceivable that the rapid growth in length of the various segments, posture, body type, heredity, changes in position of the stomach and other organs, may have considerable significance in the movements and biochemical processes of the digestive tract. As growth proceeds, adequate digestive functioning, in turn, is dependent upon increased ability of the gastrointestinal tract—to supply the larger body with greater amounts of assimilable material for expanded maintenance and growth requirements. The small intestine increases in length about 28 per cent in the first year of life although there are great variations in length of the small intestine in children, ranging from 300 to 350 cm. in the full-term, newborn child. The functional ability of the digestive tract increases during infancy. By puberty, the length of the intestine has doubled[289] and the adult functional pattern of the gastrointestinal tract has been established. Relationships between the lengths of the body, trunk, and large and small intestines make it possible to approximate intestinal growth. At birth, 9 years, and maturity, the average length of the large intestine is 66, 116 and 161 cm., respectively; of the small intestine, 338, 579 and 754 cm., respectively; of the total intestine, 404, 695 and 915 cm., respectively. Such rapid growth in the length of the digestive tract,

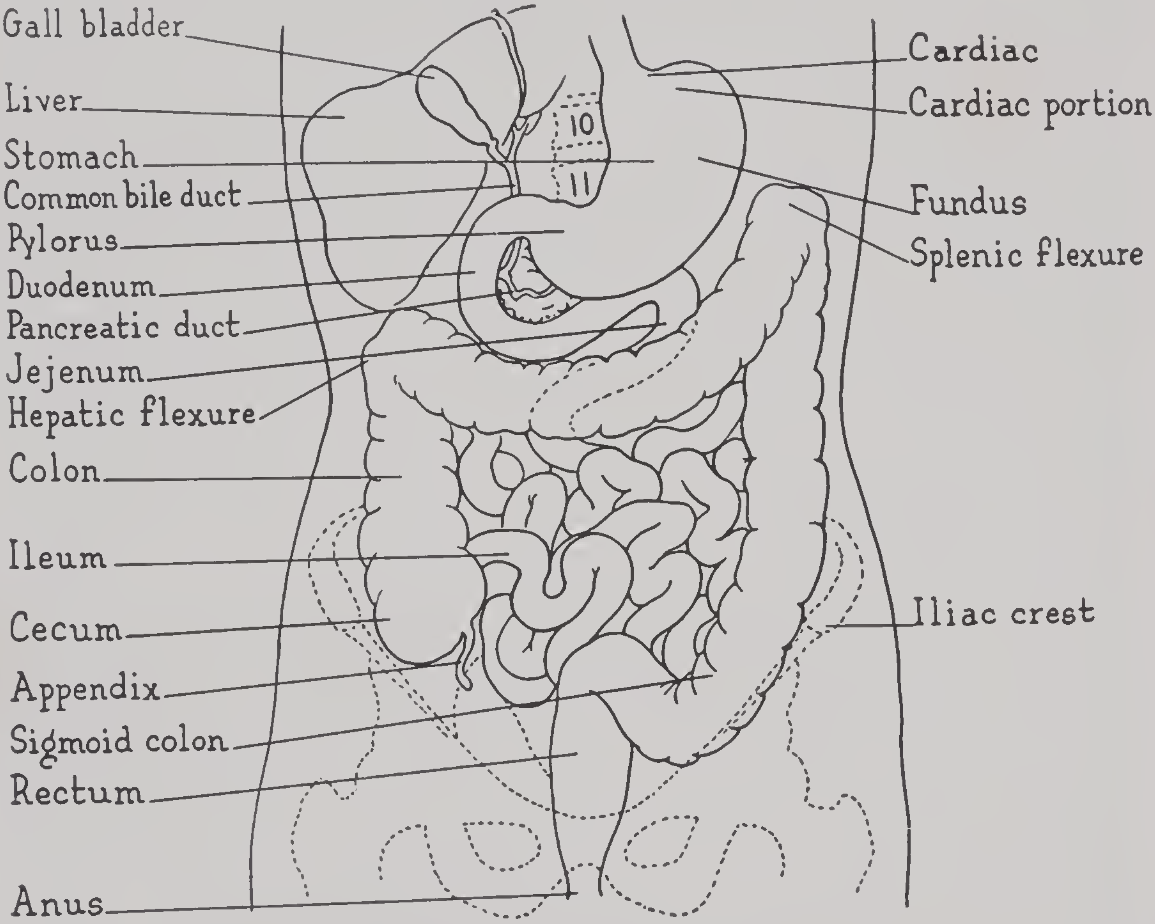

FIGURE 37. Gastrointestinal tract.

providing greater surface for exposure to digestive and absorptive processes, should be given consideration in estimating assimilation and utilization of food materials, rate of passage of food through the alimentary tract and elimination rate at different stages of growth. A detailed discussion of absorption from the intestine has been published by Verzár and McDougall.[357]

The use of radioactive isotopes has made possible a new approach to the study of the rate of absorption of foods in the body. Hamilton,[121] employing the radioactive isotopes of sodium, chlorine, bromine and iodine, has been able to detect their presence in the hand in three to six minutes after consumption, the absorption being complete in three hours. Potassium, however, did not appear in the hand for 6 to 15 minutes and absorption was not complete until after five hours. The length of the small intestine, where the greatest absorption takes place, must be an important factor in digestion and absorption. It has been shown that, in a man having only 3 feet of small intestine, absorption of carbohydrate was normal but 25 per cent of the protein and 45 per cent of the fat was lost. The fecal fat contained a large proportion of free fatty acids, indicating fairly satisfactory digestion of fat and poor absorption of fatty acids, which resulted in interference with calcium absorption.[373]

During growth, functional processes may be going on but there is, at present, no exact procedure for determining when the gastrointestinal pattern has become fully established; how much variation exists among children within narrow age ranges, even when maintained under standardized conditions of environment, habit and food consumption; nor whether there exists any significant relation between activity and the absorption of various food components. In a quest for better understanding of the physiology of the gastrointestinal tract and its activity in the normal child during growth, we have employed several procedures, and, in some measure have attempted to appraise the selective action of the bodies of the subjects. The procedures have included roentgenological examination of gastrointestinal response to test meals of barium in various media; determination of the rate of passage of food residues through the alimentary canal; and, evaluation of the relationship between the wet and dry weight and vegetable fiber contents of food intakes and fecal excretion. Roentgenoscopic (fluoroscopic) examinations permit study of the lungs, the heart shadow, the condition of the thymus, the liver and spleen; the size, shape and position of the esophagus, stomach and duodenal bulb and supplemented the procedures used to determine normality of the subjects of investigation of nutrition and chemical growth.*

The presence of gastric contents and the physiologic waves of contraction make it difficult to measure, roentgenographically, the form and position of the stomach. It is possible, however, to observe the change that takes place in the direction of the stomach's axis and therefore in its resultant position as growth proceeds. According to radiographic studies, the transverse position of the moderately filled stomach, characteristic of early infancy, gradually descends and changes over to the more usual "sink-drain" form as the child grows older, up to 11 years. The lowest portion of the stomach is found in a plane which may correspond to the level of the fourth lumbar vertebra or a point well below the iliac crest. Between puberty and maturity the stomach ascends. Wright[394] found in a study of 250 children, 6 to 15 years of age, that the position, size and shape of the stomach varies widely at all ages, but the variation is greater as puberty is approached. His observation indicates that the sink-drain type of stomach is more common in younger children and the sharp hook type in older ones. One of the most complete studies of the gastrointestinal tracts of infants has been published by the Child Research Council of the University of Colorado School of Medicine.[29]

In all of the metabolic balance investigations, activity of the entire gastrointestinal tract was indicated by the speed of passage of the carmine markers given to separate the feces corresponding to the food intakes for each balance period. The fact that in some cases carmine did cause a change in the consistency of the feces suggested that it may influence gastrointestinal activity. The question whether carmine is truly inert initiated, during the course of the investigations of the metabolism of normal children, a roentgenological study of the influence of an amount of carmine equal to that used to separate the feces of metabolic balance periods, upon the gastrointestinal response of the children to a milk-barium test meal.[221]

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* Roentgenoscopic examinations failed to show any abnormality in the chests, in the movement of either leaf of the diaphragms, in the rate or rhythm of the hearts, or in the filling of the duodenal bulb of the children. None of the children had any thymus enlargement and shadows of the liver and spleen were normal. In each child the size and contour of the esophagus, stomach and duodenal bulb were within the normal range. In one child the stomach was "J"-shaped and rather large for a child of his age; the stomachs of the other children were of the sink-drain type found by Wright[394] to be the most common in children. Although the configuration of the organs was within normal limits for all the children, variations in size, shape and position were noted.[223]

The roentgenographic series with carmine were obtained one week following control series, exposed with the same children and under the same conditions. Although the carmine decreased the gastric emptying time (average, 31 per cent), the time required for passage through the entire tract was unaltered and the results were consistent for the seven children participating in the investigation. Apparently, the effect of the carmine in speeding up the test meal in the first sections of the tract was counterbalanced by a slowing up in the ileum, cecum and colon, perhaps owing to less complete digestive action during the speeded up transit through the stomach, duodenum and jejunum.

TABLE 29

INFLUENCE OF CARMINE UPON LAXATION RATE OF 7 CHILDREN

Test meal	Total number of defecations for the seven children on successive days following ingestion of meal				Total defecations during the four days	Average laxation rate*
	1‡	2	3	4		
Milk + barium	6	10	9	11	36	1.28
Milk + barium + carmine†	7	9	12	6	34	1.21

* Bowel movements per day.
† The test meal with carmine was given one week following the control test meal.
‡ Day meal was given.

The number of bowel movements of all the subjects on the days the test meals were given, and on the four days following ingestion of each test meal (the barium-milk meal and the barium-milk-carmine meal) are shown in Table 29. No conclusive evidence of any laxative effect of the carmine is demonstrated by the total number of defecations per day or by the averages for the five-day period. There was an average elimination rate of 1.4 defecations per child per day during 26 days within which four barium-milk meals were given, only one of these preceded by carmine. The barium apparently slowed the gastrointestinal rate of the subjects, for under the same environmental conditions during a continuous metabolic study of the same children, over eight continuous months during which the subjects received no cathartics, the average number of defecations per child per day was 1.8. The lowered laxation rate during the roentgenological studies is in accord with common observations on the constipating effect of barium sulfate.[123]

While the roentgenological study demonstrated that carmine influenced the gastrointestinal response but not the laxation rate of the normal children, when ingested with a six-ounce barium-milk test meal, these effects must be considered minimal during metabolic balance studies when the carmine is given with a breakfast of mixed foods totaling two to three times the weight of the test meals and containing relatively large amounts of minerals and fiber. Given no more frequently than once in five days, with some assurance that any slight effect of a carmine marker is consistent for all the children in the experimental group, the record of many consecutive markers passing through the tract becomes a valuable and reliable aid in interpreting the metabolic balance data.

Information upon the gastrointestinal response of normal children also was obtained by roentgenoscopic and roentgenographic examinations following ingestion of test meals composed of barium in different types of media. The studies were made following the metabolic balance regime, while the children were living in the same environment and subject to equivalent influences from dietary, routine and supervision. Disturbance of the living routine by the special requirements of roentgenological observation was kept to a minimum and the different examinations were made at wide intervals to prevent any overlapping of the results. No laxatives were given or any alterations made in the diets, preceding the studies.

Rowe[286,287] and others[108] have shown that a change in gastric emptying time and colonic spasm, hyperstalsis and other irregularities in peristalsis may be due to sensitiveness of the intestinal tract to foods, therefore, the usual test meal of barium in water was employed for the first series of observations. In other series of roentgenographic studies, four types of media were employed to demonstrate the influence of a small amount of fat, meat, a large amount of fat and a weak concentration of sugar, upon the gastrointestinal activity of the children.[277] The barium meals were given in the morning and were the only food ingested between the evening meal preceding and the completion of the first day's roentgenograms. The first roentgenogram was obtained within 12 minutes after the meal was ingested and additional films were exposed at 30-minute and hourly intervals up to about five hours, in the case of the faster meals and seven hours in the case of the slower one. Throughout the period of observation at frequent intervals (four to eight hours) the children were allowed freedom of a room in which they played; however, they were under strict surveillance at all times to preclude ingestion of food or water. Additional roentgenograms were procured 24, 48, and in a few instances, 72 hours, approximately, after ingestion of the meals. The roentgenograms of the passage of the test meals of barium in five different types of media, through the tract of one child, are reproduced in Figure 38, and are typical of the group.[277]

Gastric Activity

The products of carbohydrate,[167,273] fat[272] and protein[112,355] digestion are known to have a physiological significance in controlling the rate at which these substances leave the stomach, whether through the action of humoral or nervous stimuli, or both, on the pyloric reflex mechanism. The gastric emptying times determined from 33 serial studies, comprising a total of over 300 roentgenograms of nine different children, were unlike for each of the meals given (Table 30).

TABLE 30
EMPTYING TIMES OF TEST MEALS FROM STOMACHS

Test meal of barium in	Minimum	Maximum	Mean
	hours	hours	hours
Water	1.0	2.8	1.9
Milk	1.5	4.5	3.1
Carbohydrate	3.0	4.0	3.3
Cream	2.5	6.5	4.8
Meat	3.0	7.0	5.0

It is evident that the type of vehicle or conveyor of the barium has a specific motor effect upon the egression of the meal through the pylorus, findings which substantiate those of Johnston and Ravdin[167] and Gershon-Cohen and Shay.[113] Study of the films of the milk-barium meal (2.3 per cent fat) showed an initial delay in passage of this meal through the pylorus into the small gut, resulting in an average gastric emptying time of 3.1 hours in contrast to 1.9 hours with the water-barium meal. The cream-

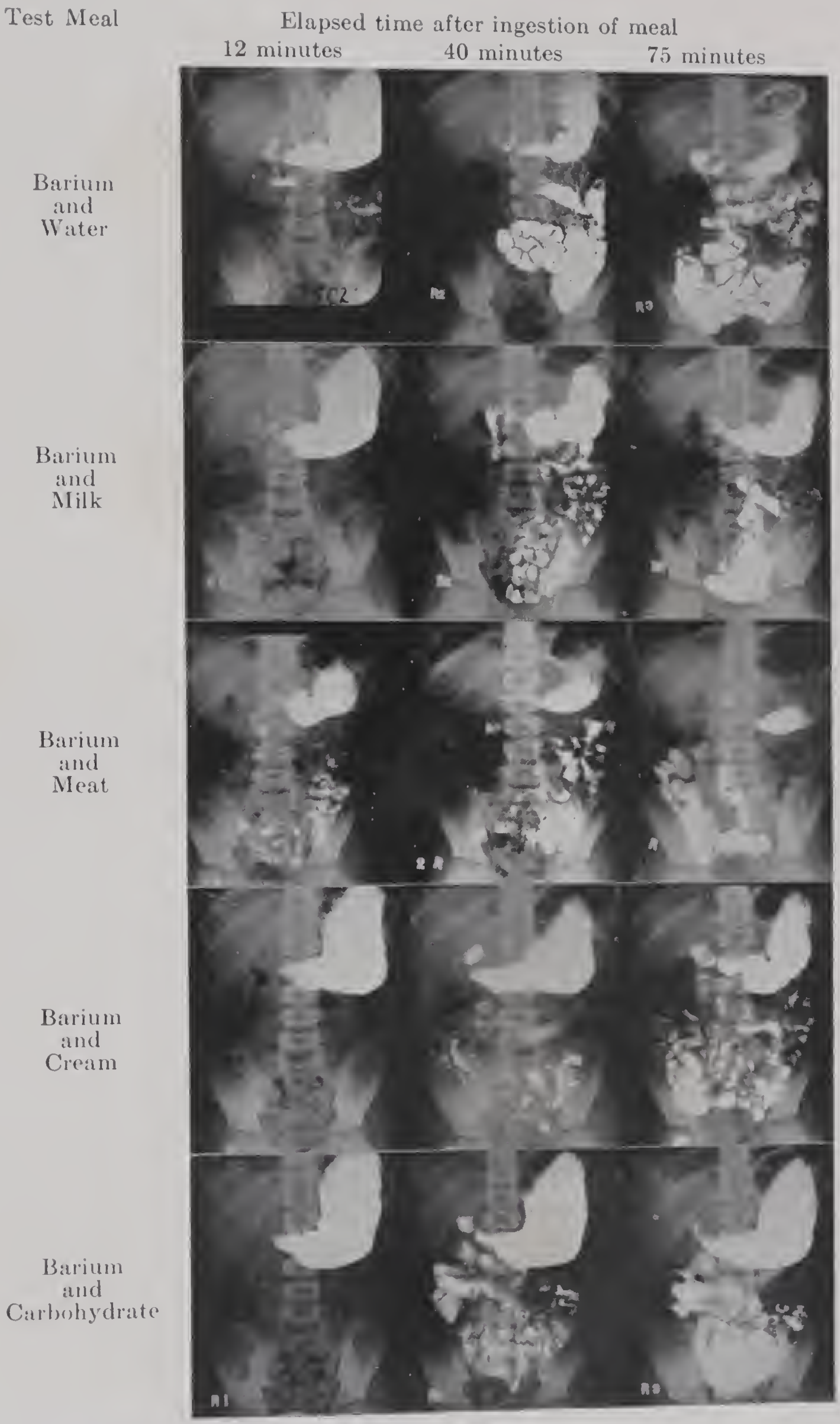

FIGURE 38. Roentgenograms of gastrointestinal tract after ingestion of test meals composed of barium and water, milk, meat, cream and carbohydrates.

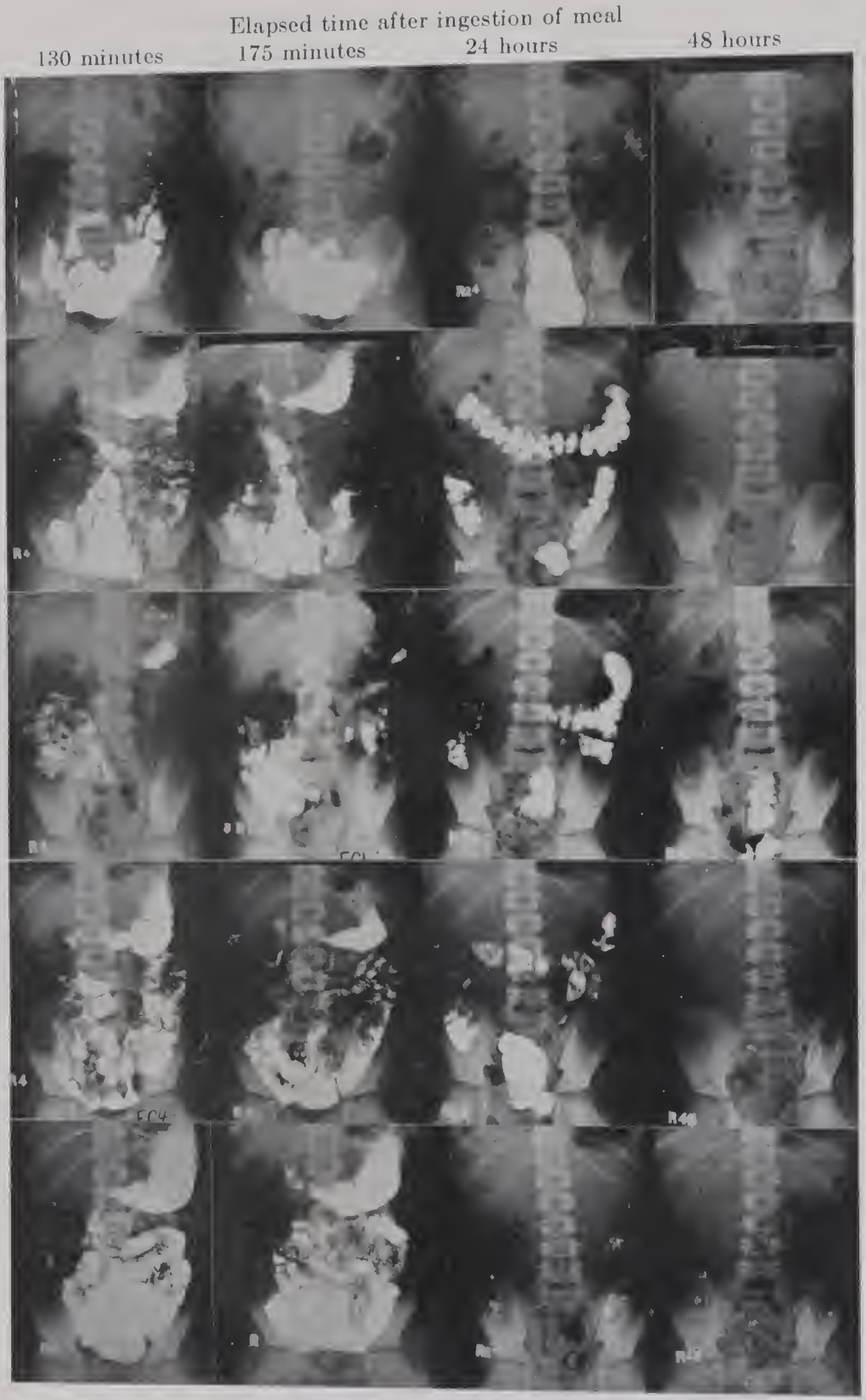

FIGURE 38. Roentgenograms of gastrointestinal tract after ingestion of test meals composed of barium and water, milk, meat, cream and carbohydrates.

—Reassembled and reproduced by courtesy of the American Journal of Roentgenology and Radium Therapy

barium meal, containing approximately 13 per cent fat, required an average of 4.8 hours to empty from the stomach (Table 30), verifying that pyloric action is controlled by the fat content of the diet.[112] The protein meal, composed of baked meat loaf accompanied by water, had a different volume and consistency than the other meals and required an average emptying time of 5.0 hours. It is known that protein requires a high gastric acidity for initial digestion. Although there is an increased acidity of the duodenum contents following a protein meal, the normal duodenum has the ability to neutralize the acids that reach it from the stomach, thus permitting almost unvarying *pH* within the jejunum and ileum.[230]

FIGURE 39. Measuring roentgenograms with the planimeter.
—*Reproduced by courtesy of the* American Journal of Diseases of Children

The carbohydrate meal produced a retardation in gastric evacuation greater than resulted with the milk meal, although no fat was present to delay stomach emptying. This is in agreement with recent experimental work upon gastric physiology[113,167,232,275] involving the influence on pyloric action of osmotic pressure changes produced by sugar solutions. The results suggest that osmotic pressure above or below isotonicity definitely influences pyloric action and gastric evacuation, and the greater the hypertonicity of the meal, the slower the gastric emptying.

The body, which is organized for homeostasis, always attempts to bring the ingested fluid to the isotonic state; consequently, when a hypertonic sugar solution is introduced into the stomach there follows a rapid increase in the volume of gastric contents and a decrease in sugar concentration. In this study the physiological homeostatic effect of the stomach, in rendering the ingested hypertonic carbohydrate fluid isotonic, can be demonstrated by a comparison of the changes in volume of the stomach contents of the same individuals when inert water and the carbohydrate are used as conveyors of the barium. Although planimeter measurement of roentgenograms showing the area of the maximum longitudinal section of the stomach has limitations (the third dimension cannot be obtained) qualitative information significant to the roentgenologist can be secured.

TABLE 31

AVERAGE CHANGES IN CROSS SECTIONAL AREA OF CHILDREN'S STOMACHS AFTER INGESTING WATER AND CARBOHYDRATE TEST MEALS*

Test meal of barium in	Size of stomach† after		
	12 minutes	40 minutes	75 minutes
Water	100 per cent	72 per cent	63 per cent
Carbohydrate Solution	128 per cent	122 per cent	117 per cent

* The instrument used was a disc planimeter made by G. Coradi, Zurich.

† All of the percentages are based upon 100 per cent as the size of the stomach 12 minutes after ingestion of the water-barium meal.

From the planimeter measurements (Figure 39) of the barium shadows of the stomachs after ingestion of the inert water-barium mixture and a meal consisting of an equal amount of the slightly hypertonic carbohydrate-barium meal (Table 31), it is obvious that, even by the time the first film could be taken, the carbohydrate meal caused a significant increase in the average size of the stomachs. The size of the stomachs increased rapidly and then decreased much more slowly with the sugar solution, and were larger after 75 minutes than with the water meal after only 12 minutes. These results explain the satiety and sense of fullness, followed by a cessation of hunger contraction and a submerging of appetite, which follow the consumption of candy or sweets at any time. It is of significance that this effect may last one and one-half hours with concentrations as low as 10 per cent carbohydrate. A few pieces of candy would have a more lasting effect.

Intestinal Activity

Experimental evidence indicates that activity of the *muscularis mucosae* keeps the food finely divided so that the digestive juices may operate, and propels the digested food along the course of the small intestine. That the peristaltic waves are seen in the small intestine during roentgenological examination only when it is somewhat overloaded with a mass of food has long been known, though the jejunal and ileal patterns are always distinct in appearance. When food approaches the region of the ileocecal valve, intestinal movements are much less active; there is considerable massing and overlapping of the shadows; and the outline of that section of the intestine is less well defined on the roentgenogram. The nearer the food approaches the distal end of the small intestine, the less peristalsis is visible. During roentgenoscopic observations, a sudden propulsion of contents from the last few inches of the ileum into the cecum is frequently seen.

In the series of roentgenograms for the children who received all of the test meals, each child demonstrated an individual emptying time and dispersion pattern for all the test meals but variations with the different meals were greater than variations in the individuals, results which are in agreement with those Ladd obtained with infants.[191] Consistently, the most rapid passage was shown by the water meal and the least by the meat and cream meals. Even the individual variations in response seemed to be controlled in the direction of producing the same final result for all the children; the subjects who tended toward faster gastric emptying than others in the group, showed slower passage through the intestines. Those whose stomachs emptied less rapidly, demonstrated faster transit through the intestines.

A study of gastrointestinal activity is productive of significant observations other than those of factors which indicate gastrointestinal response. In metabolic studies gastrointestinal activity must be considered as a possible cause of certain phenomena rather than a result. Practically all of the recent experimental work upon the nutrition and growth of normal children has added emphasis to the necessity of considering the particular individual in interpreting the results obtained with him.

CHAPTER VI

SOME CHEMICAL ASPECTS OF DIGESTION AND EXCRETION

WE have pointed out that physical well-being is dependent upon heredity and environment and that food comprises one of the most important environmental influences to which the body is subject, but nutritional inquiry cannot be fully satisfied by studies of the composition of food eaten, nor by investigation of the influence of dietary upon body size. The metabolic fate of the organic and inorganic components of food also must be studied.

While there is only one normal pathway for food consumption there are several paths of excretion, through the kidneys, gastrointestinal tract, lungs and skin. The various paths of excretion are closely interrelated and upon their proper functioning depends the well-being of the body as a whole. They are interchangeable to a limited degree—increased physical exertion may produce augmented excretion through the lungs and skin and reduce urinary losses; with diarrheal disturbances some excretion which would normally be emitted in urine may be diverted into the feces. Elevation of environmental temperature has been shown to increase water elimination through the skin and reduce the dilution of the urine.[195] Likewise, an increase in relative humidity caused similar, but less striking, changes. In neither case was the amount of fecal or retained water markedly affected.

Sweating is one of the important physiological regulatory and protective mechanisms of the body. Uniformity in the percentage heat lost in vaporization is generally recognized. About 25 per cent of the heat lost from the bodies of children is involved in the continuous vaporization of water in the cutaneous and pulmonary excretions. About 0.58 Calories are required to vaporize each gram of water from the surface of the body at room temperature. As water is eliminated through the skin it carries with it appreciable amounts of sodium, potassium and chlorine, those elements most concerned with water metabolism. These losses have been shown to be exceedingly important, by mineral balance studies of infants[336] and adults,[107] but the requirements inherent in present methods of measuring mineral losses through the skin prevent their determination over periods of more than a few hours. That retentions of sodium, potassium, chlorine and sulfur (and possibly some of the heavy metals) calculated from analyses of intake and outgo are considerably higher than the actual amounts of these elements retained, must be recognized.

Water Exchange: Water is essential for life and for satisfactory anabolism, catabolism and elimination of waste products. It may be dissipated from the body through the lungs as expired water vapor; from the skin as perspiration; through the alimentary canal as a component part of the feces; and, through the kidneys as the solvent for urinary excretory products. The quantity of water eliminated from the body depends upon the amount of water consumed; the quantity of water formed by oxidation of the hydrogen of foods, or body tissues;[3] environmental temperature and relative humidity; body temperature and amount of exercise. Under standard conditions, average temperature 22–25° C., 30–50 per cent humidity and with light clothing, 12 year old boys have been shown to lose an average of 27 per cent of their calories through vaporization of water.[68,69] Under conditions of normal activity and living, the adult displays a tendency to rid himself, by vaporization of water, of about one-fourth of the heat produced within his body.[249,250]

Seven children, ages 8 to 12 years, who lived within the same environment, consumed similar quantities of the same foods every day for 55 days, and participated in similar amounts of organized daily activity, including periods for free play, ingested an average of 1704 gm. of water per day inclusive of moisture in food. In addition to the water ingested, additional water is formed during the metabolism of foodstuffs in the body. For each 100 gm. of protein, fat and carbohydrate metabolized, about 41, 107 and 55 gm. of water, respectively, are formed. On this basis the children were each provided with an additional 281 gm. of water per day, making the total amount of water available to each child (average daily) 1985 gm. Of this amount, 882 gm. were eliminated through the kidneys, 69 gm. in the feces, leaving 1034 gm. which must have been used otherwise by the bodies, i.e., lost through evaporation from the skin and lungs or incorporated into body tissue. Considering the body two-thirds water the average daily weight gain of the children (9.90 gm.) would represent an average of only 7 gm. of water retention per day. Admittedly, the results of calculations such as the foregoing may be considered only as broad approximations which indicate the distribution in which a body disposes of the water available, but 1027 gm. of water per day lost through evaporation from the skin and lungs of the normal children is 58 per cent greater than the 600 to 700 gm., which has been estimated as the average daily loss for an adult man.

Urine Acidity: The continuous variations in acidity or alkalinity of the urine is a reflection of the continuous metabolic adjustment made by the body throughout the day and night to maintain the physiological *pH*. Acid and alkaline juices withdrawn from the body during digestion leave the system partially depleted of positive or negative minerals; the kidneys respond by excreting acid or alkali to compensate for the loss. The averages for fasting and consecutive hourly urinary *pH* determinations are given in Figure 40 for two groups of children ages 3 to 15 years; Group A composed of 42 children and living in one children's home, Group B composed of 61 children and living in a second home. Each group was made up of approximately equal numbers of boys and girls who consumed the dietaries and par-

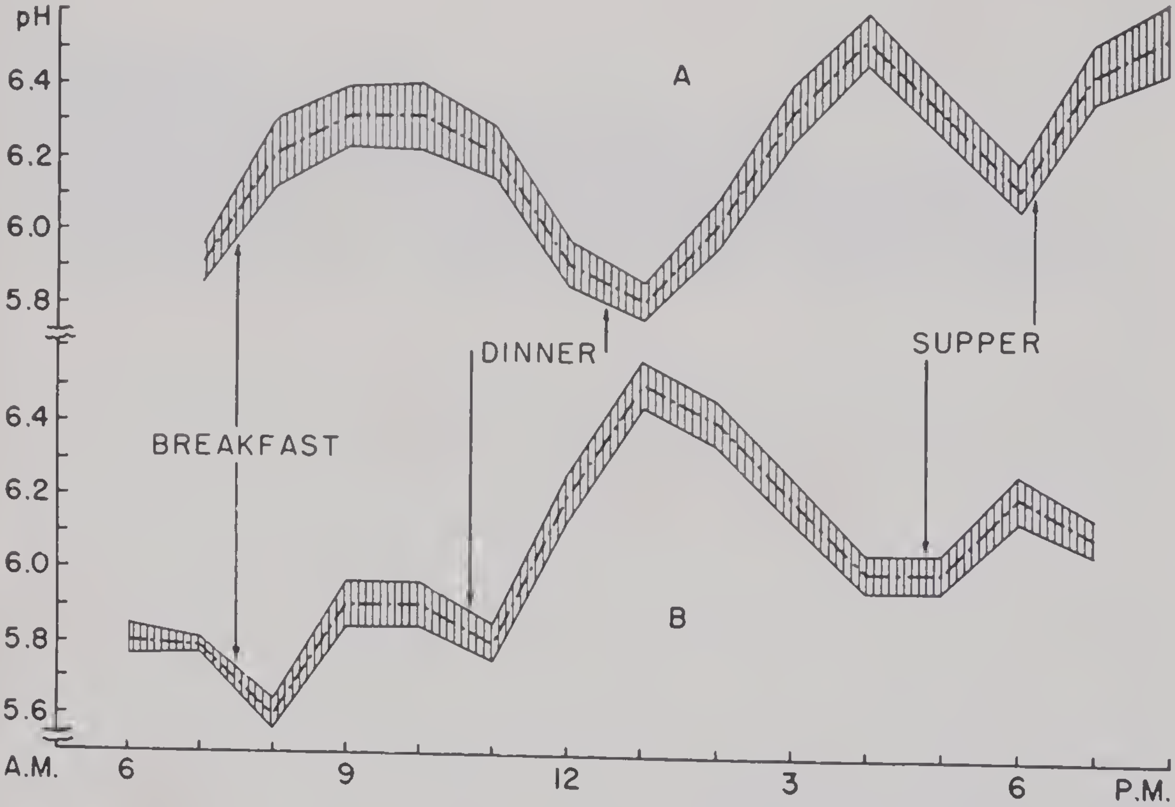

FIGURE 40. Averages of consecutive hourly *pH* determinations for two groups of children, 3 to 15 years old. Group A included 42 children, group B, 61 children.

—*Reproduced by courtesy of* Archives of Pediatrics ticipated in the usual routine of their respective institutions. Urine was collected at hourly intervals under neutral paraffin oil and toluene and the determinations made on the fresh sample after only minimum exposure of air. Distinct alkaline tides* appear in both groups after the heavy noonday meal, and alkaline trends may be observed following the lighter meals.[176] Moreover, the alkaline tide occurred one hour after breakfast and an hour after dinner, in one instance, whereas on another day it appeared two hours after breakfast and only 15 minutes after supper.

The range of 4.8 to 8.4 urinary *pH* for the 24 hours for all the same subjects is shown in Figure 41.

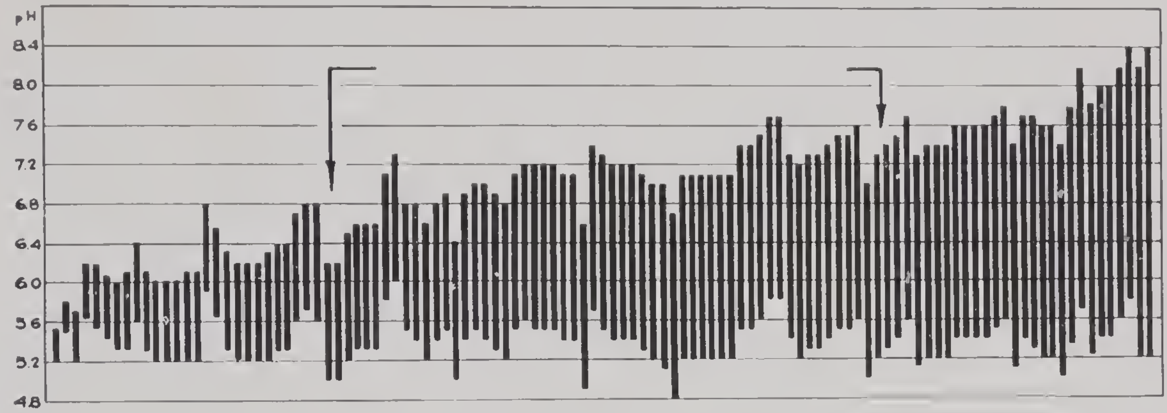

FIGURE 41. Urinary *pH* range in 24 hours. Each vertical line represents the actual extent of variation in a 24-hour study for an individual, and these lines are arranged in order of magnitude irrespective of all other factors. The arrows indicate the middle 50 per cent of the determination.

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It has been pointed out that there are differences in the emptying time of the stomach which may be due to the type, amount and temperature of the food consumed, as well as to emotional states of the individual. A difference in the emptying time of the stomach may also be reflected in the urinary *pH* values. Children show not only extensive variations in urinary *pH* through the day, but also widely different urinary *pH* levels on different days, which may change from day to day depending upon many intrinsic and extrinsic factors. One nine year old girl whose urine was examined at hourly intervals throughout the waking hours for 8 consecutive days showed practically as great a range of urinary *pH* from day to day as was shown among different individuals (Table 32).

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* Hubbard[154] considers that an alkaline tide is present if one specimen is more alkaline by 1.0 *pH* units than any samples collected previously, or if any two are more alkaline by 0.5 *pH* units than a previous specimen.

Composition of Urine

Urine is eliminated at intervals but is excreted continuously by the kidneys and collected in the urinary bladder. It is composed largely of water (about 95 per cent), but contains solids which have their origin in the surpluses of the intake and in the waste products of metabolism. The nitrogenous constituents discharged are principally, end products of protein metabolism; the mineral salts are derived from the food ingested and from the metabolic processes concerned in growth and the maintenance of the electroneutrality of the body. The composition of urine varies widely, depending upon the amount and kind of food consumed, water intake, environment, and upon the intensity of the physiological activity involved in the maintenance of the osmotic and ionic equilibria of the body.

TABLE 32

HOURLY VARIATIONS IN URINARY *pH* OF ONE GIRL DURING EIGHT CONSECUTIVE DAYS

pH after fasting*	*pH* AT TIME INTERVALS IN HOURS FOLLOWING														
	BREAKFAST				DINNER						SUPPER				
	¼	1	2	3	¼	1	2	3	4	5	¼	1	2	3	4
6.2	6.4	7.6	7.1	7.8	—	7.8	7.2	6.7	6.4	6.6	6.3	6.0	5.8	5.8	5.6
5.9	6.0	5.9	6.1	—	6.0	5.8	6.0	6.1	6.2	6.2	—	5.6	6.0	6.2	—
6.1	—	6.1	5.9	5.7	—	5.5	5.9	6.0	6.4	7.1	6.2	5.8	6.0	5.9	—
6.0	5.5	5.7	6.0	5.6	—	5.6	5.8	6.2	5.8	5.9	—	5.7	5.8	5.9	—
5.8	5.5	5.5	5.4	5.6	—	5.5	5.5	5.5	5.5	5.5	—	5.4	5.5	5.5	5.5
5.6	5.4	5.5	—	—	5.7	5.5	5.7	5.6	5.7	6.1	5.9	5.5	5.5	5.7	5.7
5.8	5.5	5.4	5.6	—	6.1	6.4	6.8	6.4	6.1	—	—	5.7	5.7	6.1	5.9
5.7	5.5	6.0	7.2	—	6.1	5.9	6.1	6.2	6.2	6.1	7.0	5.9	5.8	5.9	—

* *pH* of urine eliminated before breakfast.

Urine Volume and Concentration: The quantity of urine produced depends directly upon the total amount of water consumed and the water formed during combustion of food and body substance. Urine volume varies inversely with the amounts of excretion through the lungs, skin and intestines, which are controlled, in large part, by body activity and the temperature and humidity of the environment. The volume of urine excreted may be influenced by nervous excitement, altering its concentration of solids. The range of the average daily urine volumes of children is demonstrated by data obtained with a group of 103 normal children, between the ages of 3 and 15 years, on unrestricted diets. With approximately equal numbers of boys and girls in the group, the daily urine volumes ranged from 150 to 2100 ml. with a mean of 948 ml. Specific gravity of the urine ranged from 1.001 to 1.032, with a mean of 1.012. During the metabolic balance study of seven children, 8 to 12 years old, the average daily urine volume was 906 ml. The average distribution of water and solids in the urine of the children was 882 and 46 gm., respectively. The mean energy value of the urine was 92 Calories per day, of which an average of 51 Calories, 55 per cent, was represented by urea.

Nitrogen Partition of Urine

Among the nitrogenous end products of protein metabolism found in urine are urea, ammonia, uric acid, creatine and creatinine. The portion of the total urinary nitrogen* which is not determined in analyses for these nitrogen fractions is designated as undetermined nitrogen. The nitrogen partition of the urine of 28 children, during 1014 days, is given in Table 33 and the percentage distribution of the total nitrogen in Table 34. The calcu-

TABLE 33

AVERAGE DAILY NITROGEN DISTRIBUTION IN URINE OF 28 CHILDREN DURING 1014 EXPERIMENTAL DAYS*

Values in milligrams

Age group	Ammonia nitrogen		Urea nitrogen		Uric acid nitrogen		Creatine nitrogen		Creatinine nitrogen		Undetermined nitrogen		Total nitrogen†	
yr.	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
4	231	44	6660	1018	129	29	132	65	157	22	284	343	7593	1082
5	243	70	6718	1051	122	19	166	78	164	26	353	350	7766	1092
6	252	46	7302	1035	126	14	157	67	189	26	321	348	8347	1104
8	304	75	9168	1444	135	18	134	68	240	36	412	401	10393	1594
9	362	75	9109	857	137	17	129	29	226	26	654	289	10617	974
10	325	86	9449	1125	143	24	94	75	295	56	727	359	11033	1136
11	355	84	9513	530	151	11	45	22	319	12	636	314	11019	469
12	390	168	9343	720	148	19	75	39	350	38	736	237	11042	843

* In Table 20, page 87, are shown the distribution of the subjects among the age groups; the distribution of the five-day balance periods among the subjects; and, the average body weights and recumbent lengths of the subjects in each age group during the balance periods of study.

† Total nitrogen data are the averages of daily determinations and vary slightly from the balance figures obtained with period composites.

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* In compiling data representative of different metabolic balance periods we have used total nitrogen values obtained from analyses of the urine composites for the balance periods (p. 153). Averages of analyses of daily urine specimens for total nitrogen have been used in comparison with nitrogen partition values.

lated daily excretions of ammonia, uric acid, urea, creatine and creatinine are given in Table 35.

Of the average amounts of nitrogen excreted in urine each 24 hours by the entire group, an average of 290 ± 90 mg. were found as ammonia; 8074 ± 1597 mg. in urea; 132 ± 22 mg. in uric acid; 133 ± 74 mg. in creatine; and, 221 ± 64 mg. in creatinine (Table 33). The difference between the average daily excretion of total nitrogen by the group and the nitrogen found as ammonia, urea, uric acid, creatine and creatinine was 461 ± 382 mg. (undetermined nitrogen). That values for undetermined nitrogen include

TABLE 34

AVERAGE DAILY NITROGEN DISTRIBUTION IN URINE BASED ON A STUDY OF 28 CHILDREN

Values in percentage of total nitrogen

Age group	Ammonia nitrogen	Urea nitrogen	Uric acid nitrogen	Creatine nitrogen	Creatinine nitrogen	Undetermined nitrogen
yr.						
4	3.0	87.7	1.7	1.7	2.1	3.8
5	3.1	86.5	1.6	2.1	2.1	4.6
6	3.0	87.5	1.5	1.9	2.3	3.8
8	2.9	88.2	1.3	1.3	2.3	4.0
9	3.4	85.8	1.3	1.2	2.1	6.2
10	2.9	85.6	1.3	0.9	2.7	6.6
11	3.2	86.3	1.4	0.4	2.9	5.8
12	3.5	84.6	1.3	0.7	3.2	6.7

TABLE 35

AVERAGE DAILY COMPOSITION OF URINE OF 28 CHILDREN DURING 1014 EXPERIMENTAL DAYS*

Values in milligrams

Age group	Children studied	Daily determination	Urea	Ammonia	Uric acid	Creatine	Creatinine
yr.	number	number					
4	5	70	14268	281	387	412	422
5	5	238	14393	295	366	518	441
6	6	217	15644	306	378	490	508
8	5	157	19642	370	405	418	646
9	2	108	19515	440	411	402	608
10	3	115	20244	395	429	293	608
11	1	54	20381	432	453	140	794
12	1	55	20016	474	444	234	942

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* In Table 20, page 87, are shown the distribution of the subjects among the age groups; the distribution of the five-day balance periods among the subjects; and the average body weights and recumbent lengths of the subjects in each age group during the balance periods of study.

summation of errors in all the other determinations is shown by the large standard deviation for the average content. The presentation by age groups shows obvious trends with age and increase in body size; increases in urea, uric acid, and creatinine nitrogen and a decrease in creatine nitrogen.

Sulfur Partition of Urine

Sulfur compounds found in urine also are mainly products of protein metabolism. The sulfur of urine can be quantitated and the distribution of the total amount determined by analyses for the sulfur present as inorganic or ethereal sulfate. The excess of the total sulfur over the sum of inorganic and ethereal sulfate is designated as neutral sulfur.

The average daily urinary sulfur excretion for seven children, 8 to 12 years old, during 63 five-day balance periods was 764 ±43 mg. Eighty-four per cent (643±36 mg.) was excreted as inorganic sulfur, and six per cent (48±12 mg.) was found as ethereal sulfur. Ten per cent (73±14 mg.) of the sulfur excreted in the urine was neutral sulfur. Analyses of daily urine collections from seven children for five days yielded a check on the reliability of analyses of five-day urine composites and revealed the constancy of the sulfur partition from day to day on a constant dietary.

Neutrality of Urine

The maintenance of body fluids and tissues at the physiologically proper hydrogen ion concentration is essential to life. The cation and anion contents of the urine result from the body's efforts to maintain this neutrality. There are numerous factors that may enter into the adjustment or control of the acidity and alkalinity of urine which result from the type of diet consumed and the physiological processes accompanying its digestion, absorption and utilization. The total amount of any one component excreted during a 24-hour period depends upon the characteristic physiological make-up and growth demands of the individual child, the diet, and environmental conditions. During 55 consecutive days, seven children, while receiving constant intakes of food, excreted an average of 25 meq. of non-mineral cations (NH_4^+), 203 meq. of mineral cations, 214 meq. of mineral anions, 17 meq. of titrable acid and 33 meq. of organic acids.[131]

Positive and Negative Minerals in Urine

Although the dietary intake may be constant in composition, the excreted amounts of positive and negative minerals may vary from day to day, depending upon current metabolic demands for growth and maintenance. Together, the positive and negative minerals constituted an average of 25 per cent of the total urinary solids of the children investigated by the Laboratory. The mean excretion is given by age groups in Table 36.

TABLE 36
AVERAGE DAILY MINERAL COMPOSITION OF THE URINE*
Values in milligrams

Age group	POSITIVE MINERALS				
	Calcium	Magnesium	Sodium	Potassium	Total
yr.					
4	109	74	1729	2147	4059
5	88	83	1828	1983	3982
6	73	91	1981	2043	4188
8	122	84	2138	2364	4708
9	137	108	2691	2629	5565
10	105	110	2465	2779	5459
11	74	93	2738	2853	5758
12	159	93	2524	2878	5654

Age group	NEGATIVE MINERALS				Total positive and negative minerals
	Phosphorus	Chlorine	Sulfur	Total	
yr.					
4	642	2941	520	4103	8162
5	635	3009	525	4169	8151
6	591	3176	575	4342	8530
8	794	3428	657	4879	9587
9	897	4071	732	5700	11265
10	829	3990	731	5550	11009
11	918	4198	772	5888	11646
12	975	3824	760	5559	11213

* See Table 20, page 87 for body weights and lengths of subjects, distribution among age groups and number of balance periods for each group.

Heavy Metals in Urine

Iron, nickel, manganese, zinc and copper have been difficult to determine accurately with the chemical methods available, because these elements are present in such small quantities. For this reason, few analytical values for these elements in the urine of children appear in the literature. Iron serves an essential role in blood formation and for this reason its metabolism has been studied and emphasized. Less information is available on copper, nickel, zinc and manganese metabolism. During metabolic balance study of seven children, 8 to 12 years old, 254 five-day balances were determined for copper, nickel, zinc and manganese, in addition to those for iron. Excretion of all five elements, in urine, constituted approximately 1 per cent of the average total solids content of the urine. The average amounts of copper (0.34 mg.) and zinc (0.48 mg.) found in the urine were larger than its iron content (0.18 mg.) and portions of the manganese and nickel intakes were determined in the urine, suggesting that all of these elements play a role in the nutrition of the normal child.

Composition of Feces

The fecal material expelled from the alimentary canal consists of unused food residues, waste secretions and excretions, bacteria and their waste products, mineral salts, cell debris and water. The consistency and amount of feces excreted depends in large measure upon the character of the diet, eating habits, water intake, elimination habits, and the individual's physiological and neurological patterns. There are daily variations in the performance of the gastrointestinal tract, as well as variations among individuals of similar age, even when the same foods in like quantities are consumed each day and elimination habits, hygienic and environmental conditions are standardized.

Elimination time and laxation rate are intimately related to fecal wet and dry weights and perhaps to the chemical composition of the feces. Elimination time is determined as the interval required for specific material to pass through the tract; laxation rate is the number of defecations per day. In the investigations of normal children the average daily water intakes (including moisture in food) of 18 children, 4 to 12 years of age, ranged from 1263 to 1745 gm. during 502 five-day balance periods. During the same interval the fecal material eliminated ranged from 77 to 117 gm. per day in wet weight and from 15 to 21 gm. per day in dry weight (Table 37). Among the individuals, neither wet nor dry weights of the feces paralleled the water intakes, indicating that with this group neither the amounts nor the consistency of fecal excretion was a result of the water consumed. The average daily fecal excretion of the different children ranged from

74 to 84 per cent in water content. The average daily dry weights of the feces were also highly variable but both the wet and dry weights of the feces were within narrow ranges of percentage of their respective wet and dry weights of intake. For the entire group the wet weight of the feces averaged 5.4 per cent of the wet weight of food intake and the dry weights of the feces averaged 4.6 per cent of the dry weights of the intakes and 0.99 per cent of the wet weight of the intakes and 18 per cent of the weight of wet feces.

TABLE 37

FECAL ELIMINATION TIME, LAXATION RATES AND AVERAGE DAILY WET AND DRY WEIGHTS OF FECES OF 18 CHILDREN DURING 2510 EXPERIMENTAL DAYS*

Age group	Elimination time†		Laxation rate‡	WET WEIGHT			DRY WEIGHT				
				Feces		Of wet intake§	Feces		Of dry intake	Of wet intake§	Of wet feces
yr.	hr.	SD		gm.	SD	per cent	gm.	SD	per cent	per cent	per cent
4	19	10	2.0	84	21	5.1	15	3	4.3	0.90	18
5	18	10	2.0	109	32	6.8	18	5	5.4	1.13	16
6	30	14	1.4	84	20	5.1	16	4	4.5	0.98	19
8	29	14	1.5	100	40	5.3	18	4	4.3	0.95	18
9	25	9	1.7	105	21	4.9	20	3	4.7	0.94	19
10	38	16	1.2	77	13	3.6	19	2	4.0	0.88	25
11	21	11	1.1	117	14	5.3	21	2	4.5	0.96	18
12	50	16	0.8	77	8	3.5	20	1	4.2	0.90	26

* See Table 24, page 92 for body weights and lengths of subjects, distribution among age groups and number of balance periods for each group.

† Average time required for carmine marker to traverse tract.

‡ Bowel movements per day.

§ Wet intake includes drinking water.

Since laxation rate is so often influenced by the retention of feces over the long night interval, data on the speed of passage of a test meal through the alimentary canal assists in expressing gastrointestinal motility. Elimination time has been judged by the number of hours elapsing between the ingestion of a carmine marker and its appearance in the feces (see p. 25). Urinary ethereal sulfate plotted against the empirical ratio of time of food passage to the number of bowel movements per day shows a high correlation (Figure 42). On the basis of observations on the relation of urinary ethereal sulfate output with the food passage time it has been found that an elimination time of 36 hours or less is associated with low excretion of ethereal sulfate; longer elimination times are accompanied by a steep rise in the elimination of ethereal sulfate. The ethereal sulfate similarly shows a significant correlation with the total fecal dry weight and disappearance by fermentation of the ingested cellulose and hemicellulose from the gastrointestinal tract, despite the fact that the digestive enzymes of man are incapable of splitting cellulose and hemicellulose. Greater amounts of unavailable carbohydrate are lost in slow gastrointestinal tracts and the amount which disappears is related to the extent of fermentation due to bacterial action. The subjects having a higher dry weight of feces and lower output of cellulose and hemicellulose, which indicates longer fecal retention and a more extensive exposure to bacterial action, show higher levels of ethereal sulfate excretion. This is another indication that the level of ethereal sulfate output is related to fecal retention and putrifactive bacterial action.

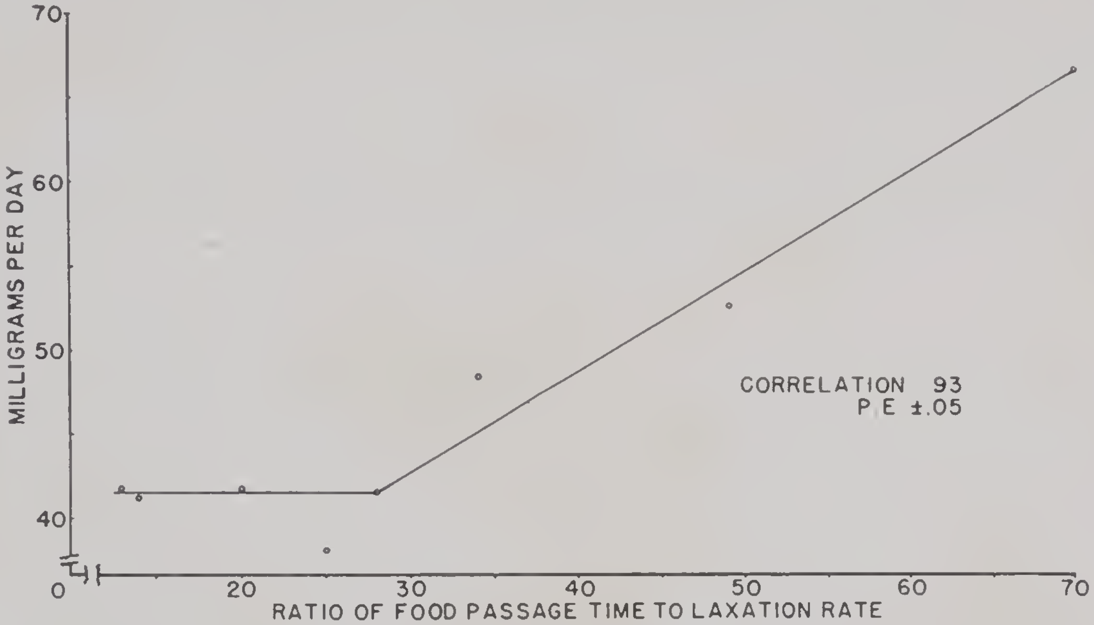

FIGURE 42. Urinary ethereal sulfate plotted against the ratio of food passage time (elimination time) to number of bowel movements per day (laxation rate). Each point represents the average for one child, over 45 consecutive days.

Ethereal sulfate is believed to have its origin in the phenol, indol and related compounds that arise from bacterial decomposition or putrefaction in the intestine. These substances are conjugated with sulfuric acid and excreted as the potassium salt. How many of the ill effects of ordinary constipation can be attributed to the absorption of toxic products of putrefaction is a question which has not been satisfactorily answered. In the present study, the daily ethereal sulfate excretion plotted against the laxation rate (the number of bowel movements per day) shows a definite relationship (Figure 43). The average daily output of ethereal sulfate for each five days is plotted against the average daily number of bowel movements for the same five-day period; this was done for seven children over a period of 55 continuous days. The average curve shows that a laxation rate of more than 1.0 to 1.2 bowel movements per day does not materially decrease the ethereal sulfate output but that a slower rate results in a rapid increase in the output of detoxication compounds. The output of 42 mg. of ethereal sulfate sulfur is fairly constant for all subjects having one or more bowel movements per day.

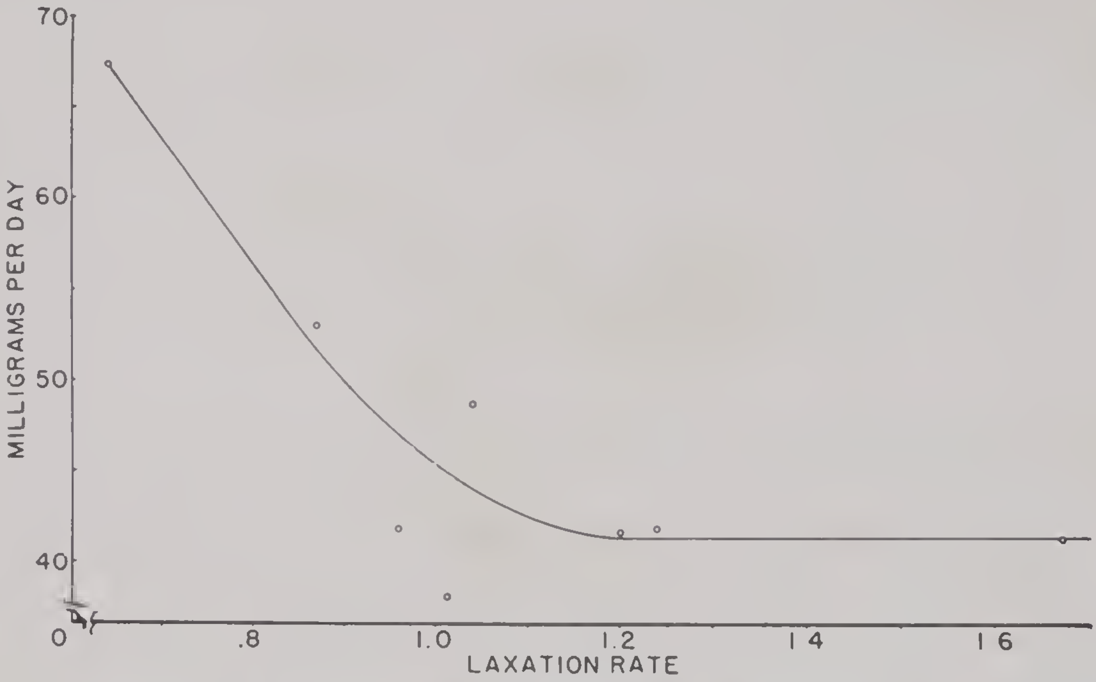

FIGURE 43. Urinary ethereal sulfate plotted against laxation rate.

Fecal Fat: The feces always contain a measurable amount of lipid material which consists mainly of fatty acids and their salts (soaps), sterols and a small amount of neutral fat or triglyceride. Infants and children are not as efficient as adults in their digestive processes and therefore their feces may contain some fecal fat of exogenous origin. Fecal fat is derived from undigested food fat, bacteria and cellular debris of the alimentary canal and fats excreted into the intestine. It is now generally agreed that, under ordinary conditions, the adult human, when ingesting a normal mixture of food and even liberal quantities of fat, completely digests and absorbs the food fat.[138] Fecal lipids are largely of endogenous origin and should not be regarded as unabsorbed fragments of the diet.[188] Some investigators choose to study fat metabolism through specific analytical determinations on the feces, others by the metabolic balance method.*

TABLE 38

AVERAGE DAILY FAT CONTENT OF FECES OF 18 CHILDREN DURING 2510 EXPERIMENTAL DAYS*

Age group	FECAL DRY WEIGHT			FECAL FAT	
	Fat-free	Fat	Total	In fresh feces	In dried feces
yr.	gm.	gm.	gm.	per cent	per cent
4	13.9	1.6	15.5	1.9	10.3
5	15.6	2.1	17.7	1.9	11.9
6	14.0	2.0	16.0	2.4	12.5
8	15.6	2.7	18.3	2.7	14.8
9	17.3	3.0	20.3	2.8	14.8
10	15.0	3.7	18.7	4.8	19.8
11	18.2	3.0	21.2	2.6	14.2
12	16.0	3.8	19.8	4.9	19.2

* See Table 24, page 92 for body weights and lengths of subjects, distribution among age groups and number of balance periods for each group.

The fecal fat content may be notably increased during pancreatic disease which results in failure to supply lipase to the digesting food residues in the small intestine. When the bile, with its lipolytic properties, fails to reach the intestine in amounts sufficient to exert its normal action as a solvent for fat derivatives, digestion and alimentary absorption are impaired.[303]

The average daily fat content of the feces of 18 children during 2510 experimental days is given in Table 38. The average daily fat excretion of all the children, 2.3 gm., represented 2.4 per cent of the average daily fecal wet weight and 13.3 per cent of the fecal dry weight. The major portion of the two to four grams of fat which the children excreted daily was composed of soaps—over 50 per cent. The unsaponifiable fraction, consisting mainly of sterols, constituted approximately one-third of the total fecal lipid. The remainder of the fecal fat was almost equally divided between free fatty acids and neutral fats, each amounting to approximately 8.5 per cent of the total.

Fecal Nitrogen: Fecal nitrogen arises from cellular debris sloughed from the gastrointestinal tract, and secretions and excretions[292] into the intestine, in addition to nitrogen in undigested food residue and that which has been incorporated into the bodies of symbiotic bacteria living in the intestine. Lusk[206] has reported, from the experiments of Lissauer that an average of 8.7 per cent of the total solids of the feces is bacteria and Rubner's nitrogen value of bacteria as 0.114 gm. per gram dry bacteria. Shohl[312] has stated that one-fourth to one-half of the total fecal solids is bacteria and that about one-half of the total fecal nitrogen is bacterial nitrogen. The average values determined for the total solids and total nitrogen of the feces of normal children are given, by age groups, on a daily basis in Table 39. Also shown are the data for fecal bacteria and bacterial nitrogen, calculated according to the relationships given by Lusk and Shohl.

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* Only a trace of fat can be found in the urine under normal conditions of health.

TABLE 39

AVERAGE DAILY TOTAL SOLIDS, TOTAL NITROGEN AND BACTERIAL NITROGEN OF FECES OF 18 CHILDREN DURING 2510 EXPERIMENTAL DAYS*

Values in grams

Age group	Fecal Excretion		Fecal Bacteria†		Bacterial Nitrogen#		
	Total solids	Total nitrogen	Lusk‡	Shohl§	Lusk+	Shohl¶	Shohl£
yr.							
4	15.49	1.07	1.35	3.87	.15	.54	.44
5	17.67	1.07	1.54	4.42	.18	.54	.50
6	15.95	1.06	1.39	3.99	.16	.53	.45
8	18.26	1.14	1.59	4.56	.18	.57	.52
9	20.33	1.39	1.77	5.08	.20	.70	.58
10	18.66	1.18	1.62	4.66	.18	.59	.53
11	21.25	1.42	1.85	5.31	.21	.71	.60
12	19.82	1.43	1.72	4.96	.20	.72	.56

* See Table 24, page 92 for body weights and lengths of subjects, distribution among age groups and number of balance periods for each group.

† Lissauer, as reported by Lusk,[2 6] p 55: "In man the minimum quantity of bacteria composing the stools was 2.53 per cent, the maximum 13.54 per cent and the average was 8.7 per cent of the total solids." Shohl,[312] p. 85: "Of this [solid matter] one-fourth to one-half represents the bodies of living and dead bacteria."

‡ Total fecal solids ×.087.

§ Total fecal solids ÷4.

Lusk,[206] p. 55: "Rubner has calculated that one gram of dry bacterial substance contains 0.114 gram nitrogen." Shohl,[212] p. 85: "The feces normally contain about 1.5 gm. of nitrogen per day; about half of this is bacterial nitrogen, . . . "

+ Fecal bacteria was calculated according to Lusk ‡×0.114.

¶ Bacterial nitrogen as calculated according to Shohl: Fecal nitrogen ÷2.

£ Fecal bacteria as calculated by Shohl § multiplied by Rubner factor of 0.114 gm. nitrogen per gram bacteria.

Using the average value of 8.7 per cent of total solids as bacteria and 0.114 gm. of nitrogen per gram bacteria, the average bacterial nitrogen content of the feces of all age groups amounts to approximately 15 per cent of the total nitrogen found in the feces by analysis. If the same factor (0.114) is applied to one-fourth the total fecal solids the bacterial nitrogen values are slightly under one-half of the total nitrogen content. Assuming that the estimate of bacterial nitrogen forming one-half of the total nitrogen (Shohl) is approximately correct, then the bacterial nitrogen content of the feces of these children was about one-thirtieth of the total solids.

Vegetable Fiber

There are no known enzymes produced by the body which break down the complex carbohydrates. The bacteria in the digestive tract, however, may decompose as much as 80 to 85 per cent of the food hemicellulose and cellulose, depending upon the length of time it remains in the digestive tract, the fineness of the food particles, the maturity of the vegetable fiber and the number and kind of bacteria present. The complex carbohydrates which have escaped bacterial decomposition are excreted in the feces and have been estimated to compose 29 per cent of the total fecal solids. The excretion of lignin, cellulose and hemicellulose by the children whose nutrition and chemical growth were studied is shown in Table 40. Williams and Olmsted[388] suggest that it

TABLE 40

AVERAGE DAILY COMPLEX CARBOHYDRATE CONTENT AND HEAT OF COMBUSTION OF FECES OF 18 CHILDREN DURING 2455 EXPERIMENTAL DAYS*

Age group	LIGNIN PLUS CELLULOSE PLUS HEMICELLULOSE		HEAT OF COMBUSTION		
	Mean	Of total solids	Feces	Complex carbohydrates of feces	
			Mean	Mean†	Of fecal energy
year	gm.	per cent	Cal.	Cal.	per cent
4	3.10	20	71	13	18
5	3.69	21	79	15	19
6	3.22	20	73	13	18
8	3.14	17	88	13	15
9	2.90	14	111	12	11
10	2.43	13	100	10	10
11	2.71	13	113	11	10
12	2.58	13	111	10	9

* See Table 24, page 92 for body weights and lengths of subjects, distribution among age groups and number of balance periods for each group.

† Weight of lignin, cellulose and hemicellulose ×4.1.

is the cellulose and hemicellulose that ferments and disappears during passage through the intestine which influences the volume of feces, and that there may be a relationship between this disappearance and the amount of volatile fatty acid excreted in the feces. Unfortunately the analysis of the wet stools of the children for volatile fatty acid was not carried out. However, since most of the free fatty acids are thought to arise from the fermentation of carbohydrate, and have been shown to be relatively independent of changes in the fat content of the diet, correlations between the amount of free fatty acid excreted and the amount of cellulose and hemicellulose disappearing in the tract have been made. In only one case was there a positive correlation; however, when the free fatty acid excretion is correlated with the amounts of cellulose and hemicellulose excreted in the feces, a positive result was obtained for ten out of eleven cases for cellulose and in every case for hemicellulose.

TABLE 41

AVERAGE DAILY COMPLEX CARBOHYDRATE CONTENT OF FECES OF 18 CHILDREN DURING 2455 EXPERIMENTAL DAYS*

Values in grams

Age group	Lignin	Cellulose	Hemicellulose
yr.			
4	1.83	0.50	0.77
5	1.76	0.88	1.05
6	1.62	0.65	0.95
8	1.53	0.71	0.90
9	1.34	0.85	0.71
10	1.27	0.55	0.61
11	1.27	0.75	0.69
12	1.42	0.56	0.60

* See Table 24, page 92 for body weights and lengths of subjects, distribution among age groups and number of balance periods for each group.

Williams and Olmsted[389] have found that hemicellulose is more effective than cellulose in increasing the bulk of the stool. Using their method for separating the vegetable fiber into its component parts the average daily fecal excretion of lignin and cellulose and hemicellulose for 18 children 4 to 12 years old, over 2455 days, is given in Table 41. In the passage of the total cellulose and hemicellulose through the digestive tract an average of 3.22 gm. of the total intake of 4.84 gm. was broken down and disappeared. Although the cellulose and hemicellulose of these particular diets were broken down in the alimentary tracts of these children we were unable to determine with methods and observations at our disposal whether the energy represented by decomposition of fiber (four to eighteen Cal. per day) was nutritionally available to the children. It is obvious that such a small number of Calories does not contribute significantly to the energy exchange of man. There was wide individual variation among the children, which confirms the statement of Mangold[229] that "The digestibility of fiber in man is extremely variable, and consequently the experimental results of different workers . . . can scarcely be compared and used to give averages valid for the general case." Mitchell and Hamilton[238] and Schneider[292] have shown that the amount of endogenous nitrogen excreted in the feces of the adult depends to a large extent upon the total intake of dry food and the body weight of the individual.

Carbon

As much as 13.5 gm. of carbon per day may be ejected in the feces. In 66 balances with seven children, 8 to 12 years of age, the average carbon content of the fecal material was 9.5 gm. per child per day, 292 mg. per kilogram of body weight.

Heavy Metals

Few determinations are available on copper, zinc, and manganese contents of feces or on average fecal excretion of these elements by healthy children, although all may be important in blood formation or the enzyme activities of the body. The iron content of feces has received greater attention. The major portions of the heavy metals excreted by normal children escape in the feces. During metabolic balance investigation of seven children between the ages of 8 and 12 years, the average daily fecal excretion of copper, manganese, nickel, zinc, and iron, together, approximated 23.5 mg.; of which approximately 10.5 mg. represented zinc and 8.7 mg. represented iron excretion. While the total fecal excretion of the five elements is less than one-quarter of one per cent of the total fecal solids, evidence recently presented and experimental work now in progress indicate that these less well-known minerals play an extremely important part in the nutrition and chemical growth of children.

Positive and Negative Minerals

Fecal excretion may contain nutrient materials which have been discarded by reason of the body's selective action, in addition to some food materials that have escaped digestion, as well as intestinal secretions and excretions. The tract may aid in maintaining the electroneutrality of the body by favoring assimilation and secretion of the most needed minerals from the diet. The positive and negative minerals, together, compose approximately 10 per cent of the total weight of the fecal solids; an average of about seven per cent of the total weight of the fecal solids consists of the positive minerals. Calcium, magnesium potassium and phosphorus compose the greater part of the minerals eliminated in feces but relatively small amounts of sodium, sulfur and chlorine are present. The mean daily fecal excretion of positive and negative minerals, by normal children, is shown in Table 42.

TABLE 42

AVERAGE DAILY MINERAL COMPOSITION OF FECES OF 29 CHILDREN DURING 2965 EXPERIMENTAL DAYS

Values in milligrams

Age group	POSITIVE MINERALS				
	Calcium	Magnesium	Sodium	Potassium	Total
yr.					
4	503	168	41	311	1023
5	607	146	54	404	1211
6	596	162	34	383	1175
8	839	174	46	339	1398
9	646	158	26	331	1161
10	700	182	32	518	1432
11	711	185	58	350	1304
12	618	178	19	263	1078

Age group	NEGATIVE MINERALS				Positive plus negative minerals
	Phosphorus	Chlorine	Sulfur	Total	
yr.					
4	311	56	78	445	1468
5	355	63	86	504	1715
6	398	50	77	525	1700
8	431	58	92	581	1979
9	423	32	135	590	1751
10	527	38	120	685	2117
11	461	58	133	652	1956
12	395	26	137	558	1636

CHAPTER VII

ENERGY METABOLISM IN CHILDHOOD

IN THE early days of nutrition as a science, emphasis was concentrated upon the energy provided by different assortments of foods in diets. Later, the question of the relative value of the energy sources, protein, fat and carbohydrate, was intensively investigated. Next came the vitamin discoveries and later, analyses of proteins focused attention upon the chemical structure of nutritive substances. Recent years have witnessed an era of "enzymatic oxidative catalysts" in nutrition, with emphasis placed upon the chemical composition of the vitamins and the activities of amino acids, fatty acids (particularly in compound lipids such as phospholipids) and heavy metals in energy metabolism and other body processes. Through the biological application of organo-metallic compounds, our understanding of biological processes is being advanced.[114] Within the time used to accumulate the data which forms the basis of the present study (1930—) new catalysts have been discovered and vitamins synthesized which were before that time only vaguely known. While attention in recent research is directed to the new fields of vitamin and enzyme investigation, much of the evidence serves only to indicate the modes in which energy (the long recognized primary principle) is derived from food materials and enters into body economy.

The tempo of change in the human body is a relative concept and may not be proportional to chronologic time. Wetzel[374] recently has emphasized that in practical work with children we are required to deal explicitly with change in size, which is a secondary attribute of growth, rather than in actual changes in growth itself, this distinction being especially important for the phenomena which touch immediately or remotely upon metabolism and nutrition. Previous sections have emphasized that growth of the structures and maintenance of the physiological functions of the body require that many diverse kinds of food materials be included in the diets of children if health and well-being are to be retained. Even for individuals within the group called "normal," nature does not insist upon the same level of performance nor bestow the same limit of capacity for growth and development.

Basal Heat Production

The determination of the energy expended by the protoplasm of the body when all of the modifying factors of activity, food and environmental temperature are producing their minimal effect has become an integral part in the assay of the health status of an individual.[247] This determination of the basal or minimum heat production of muscles and internal organs of the body includes that expended by the involuntary muscular activity of breathing, of the heart in the resting state and of the smooth muscles of the alimentary canal. The test records the performance of the body at a specified time, under a given set of conditions which cannot be exactly duplicated on other occasions, because of physiological changes in the body. The relative proportions of active (muscular, glandular, nervous) and inactive (fat, bone, water) tissue in the body vary greatly with different individuals and may vary in an individual at different times,

TABLE 43

AVERAGE OXYGEN CONSUMPTION AND TOTAL HEAT PRODUCTION PER 24 HOURS

	CHILDREN				Basal tests made	Oxygen consumption†	Total heat production
	Age	Number	Recumbent length	Weight*			
	yrs.		cm.	kg.	no.	liters	Cal.
BOYS	4	1	109.2	18.3	22	203.7	983
	5	1	116.3	19.3	21	202.7	978
	6	2	120.9	22.2	42	209.3	1010
	8	4	129.9	26.5	44	222.2	1072
	9	1	140.9	29.4	4	303.0	1462
	10	2	142.2	32.8	8	281.4	1358
	11	1	138.3	35.9	4	276.9	1336
	12	1	159.0	41.1	4	297.6	1436
GIRLS	5	2	109.4	18.7	40	188.4	909
	6	2	118.4	22.1	40	192.3	928
	9	1	131.2	28.1	4	271.3	1309

* Nude.

† Standard temperature and pressure.

however, in the present study, differences of not more than 5 per cent were found in basal metabolic rate tests made within a seven-day period.

The records of oxygen consumption and basal heat production, presented in Table 43, represent 233 observations made on 18 individuals at different age levels. They are presented to emphasize this physiological phase as an integral part of the whole problem of evaluation of growth and nutrition. The longitudinal type of study, which has recently been defined as comprising more than an observation on a single individual, has added value over the usual cross-sectional type or the collection of single observations on many individuals. Indeed, Wilson[338,391] has estimated that measurements made on each of 10 children once a year for 16 years, a total of 160 observations, would give as much knowledge about the way children grow, or more, than one observation on each of 2500 children. Comprehensive studies have been published by Talbot, Wilson and Worcester,[338] by Lewis, Kinsman and Iliff,[197] Talbot[339] and by Benedict.[11,12]

The present data should have added value in that the children were exposed to the same environment, to the same nutritively acceptable dietaries and were highly trained in their performance as subjects in physiological investigations. For these reasons the interindividual variability (the deviation of the observed metabolism of each individual) and the intraindividual variability (the fluctuations of the values of the observed metabolism among individuals of the same sex and age) should be at a minimum, and since the tests were made at the same hour of the day, the intradaily variability should also be small. Some children experience stages of very rapid growth which are coincidental with an elevated basal heat production. The oxygen consumption and heat production of the nine year old children, a boy and a girl (Table 43), illustrate this situation.

Investigators have recommended various means of interpreting oxygen consumption, and the resultant heat production. Some have recommended the interpretation of the results on the basis of age and height, others include weight and still others recommend a combination of height and weight such as surface area. In the scatter diagrams in Figure 44 basal heat production is plotted against age, length, weight and surface area for 10 children whose basal metabolism was determined two and three times per month for eight consecutive months. These demonstrate a positive relationship between basal heat production and each of four physical characteristics, age, height, weight and surface area.

Muscular Response: Howell[152] in discussing the chemical theory of fatigue defines fatigue as a more or less complete loss of irritability and contractibility brought on by sustained functional activity. He points out that even when the fatigue is complete and the muscle fails to respond to maximal stimulation, a very short interval of rest is sufficient to bring about some return to irritability but for complete restoration to its normal condition a long interval of time may be necessary. It has been demonstrated[30] that a child may be taller after an afternoon rest than he is at the beginning of the rest period; in other words, the child

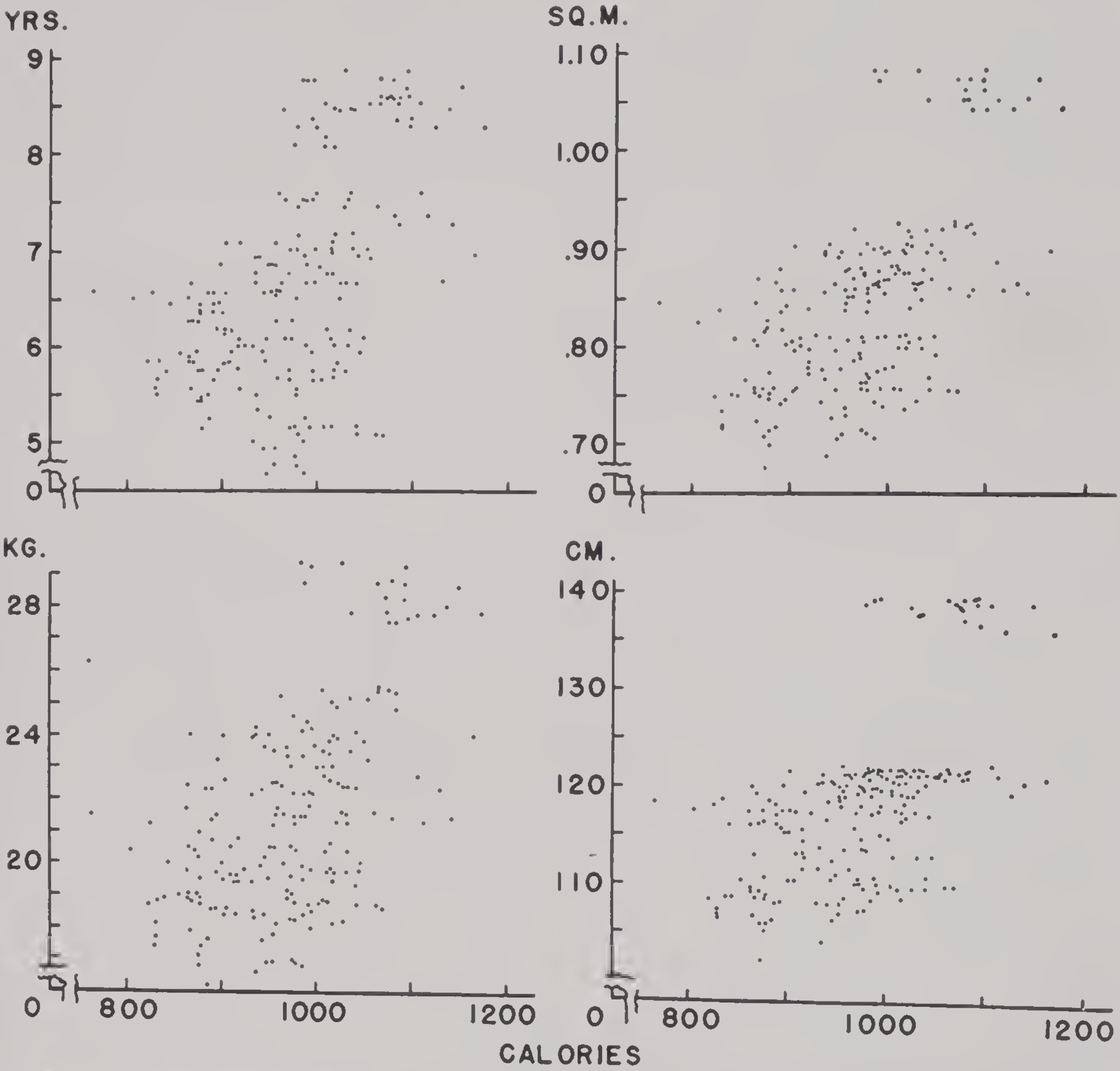

FIGURE 44. Basal heat production (Calories per day) plotted against age, surface area, weight and recumbent length.

shrinks in height during his active periods of play. Some children show a greater shrinkage than others. If this characteristic is associated with muscle fatigue, should the child not show a much greater difference between evening recumbent length, after the day's activity, and that observed at the beginning of the day after there has been a complete restoration of the muscle irritability and tone and a return to normal condition after a full night's rest? The degree of fatigue may depend upon the oxygen consumed during metabolism in the body. The daily shrinkage evidence by 152 morning and 152 evening recumbent length measurements of 10 school children is shown in Table 44.

TABLE 44

AVERAGE DAYTIME SHRINKAGE IN RECUMBENT LENGTH FOR SIX BOYS AND FOUR GIRLS, AGES FOUR TO EIGHT YEARS

Measurements in centimeters

	RECUMBENT					
	Stem length			Total length		
	Boys	Girls	Boys and girls	Boys	Girls	Boys and girls
Number of morning and evening measurements	89	63	152	89	63	152
Mean shrinkage	1.57	1.55	1.56	1.54	1.37	1.47
Standard error (SE_M)	.04	.06	.04	.04	.05	.03
Standard deviation	0.40	0.50	0.44	0.41	0.38	0.41
Standard error ($SD \pm SE_{SD}$)	.03	.04	.02	.03	.03	.02
Ninetieth percentile	2.07	2.30	2.16	1.97	1.84	1.94
Minimum	0.64	0.40	0.40	0.79	0.64	0.64
Maximum	2.86	2.94	2.94	2.54	2.38	2.54

Total Energy Exchange

The physiological fuel value of the diet is a measurement of the utilizable energy for all of the metabolic processes of the body. Determination of the heat of combustion of feces and urine measures the latent or potential energy of the material, under conditions comparable to those which exist when the heat of combustion is determined for the dietary. Subtraction of the heat of combustion of the outgo (urine+feces) from that of the intake provides an estimate of the physiological fuel value of that diet to the individuals studied, i.e., the energy (determined as heat) which has been derived from food, expended in the conduct of physiological activities and laid down in tissue. The selective ability that the body may exert through regulatory mechanisms is apparent in its control of the ways in which energy is utilized.

TABLE 45

AVERAGE PHYSIOLOGICAL FUEL VALUES OF DAILY FOOD INTAKE OF 18 CHILDREN DURING 2440 EXPERIMENTAL DAYS*

Values in Calories

Age group	HEAT OF COMBUSTION						PHYSIOLOGICAL FUEL VALUE†		
	Intake		Urine		Feces				
year	mean	SD	mean	SD	mean	SD	mean	SD	per cent
4	1813	112	63	6	71	15	1679	108	93
5	1723	110	62	11	79	22	1582	100	92
6	1863	143	67	5	73	17	1723	134	92
8	2178	159	80	10	88	22	2010	138	92
9	2174	96	88	3	111	13	1975	102	91
10	2495	113	94	9	100	15	2301	102	92
11	2499	102	89	8	113	12	2297	93	92
12	2499	113	99	10	111	7	2289	112	92

* See Table 24, page 92 for body weights and lengths of subjects, distribution among age groups and number of balance periods for each group.

† Heat of combustion of intake minus heat of combustion of outgo (urine + feces).

In Tables 45, 46, 47, the average daily energy exchange of 18 children is shown by age groups. For the 18 children the average

TABLE 46

AVERAGE PHYSIOLOGICAL FUEL VALUE OF DAILY FOOD INTAKE OF 18 CHILDREN DURING 2440 EXPERIMENTAL DAYS*

Values in Calories per kilogram of body weight

Age group	HEAT OF COMBUSTION						PHYSIOLOGICAL FUEL VALUE	
	Intake		Urine		Feces			
year	mean	SD	mean	SD	mean	SD	mean	SD
4	100.75	4.64	3.48	.38	3.94	.68	93.33	4.55
5	92.44	4.58	3.34	.41	4.24	.99	84.86	4.46
6	85.51	4.97	3.07	.24	3.35	.62	79.09	4.69
8	83.01	7.74	3.04	.35	3.38	.83	76.59	7.07
9	76.09	3.84	3.07	.17	3.89	.44	69.13	3.88
10	76.31	3.51	2.88	.14	3.07	.38	70.36	3.65
11	69.91	2.63	2.49	.10	3.17	.21	64.25	2.66
12	59.73	2.27	2.37	.19	2.65	.24	54.71	2.33

* See Table 24, page 92 for body weights and lengths of subjects, distribution among age groups and number of balance periods for each group

daily gross energy value of the dietaries was 1956 Cal. The mean heat of combustion of the outgo (urine+feces) was 153 Cal., approximately eight per cent of the intake, almost equally divided between the urine and feces.

TABLE 47

AVERAGE DAILY ENERGY VALUE OF URINE AND FECES OF 18 CHILDREN DURING 2410 EXPERIMENTAL DAYS

Age group	Per cent of energy intake		Outgo	Per cent of outgo	
	Urine	Feces		Urine	Feces
year			mean Cal.		
4	3	4	134	47	53
5	4	4	141	44	56
6	4	4	140	48	52
8	4	4	168	48	52
9	4	5	199	44	56
10	4	4	194	48	52
11	4	4	202	44	56
12	4	4	210	47	53

Energy Requirement

While the organism carries on the processes of maintenance, or growth and maintenance, regulatory mechanisms continually control all activities so that body temperature is constantly maintained within a very narrow range. Oxidation processes provide all of the energy expended by the body; whether the energy is employed to maintain body temperature, to support muscular activity, or to convert food substance into suitable form and incorporate it into the body tissues as increment (growth) or replacement (maintenance). Even the energy required to eliminate surplus, used, or unusable substances discarded from the body, is included in the measurement of physiological fuel value. Although all of the energy requirements are recognized, it is not a simple matter to determine to what degree all are met in the run-about child.

There may be sufficient energy provided in the food, but it may not be fully utilized unless specific nutrients are present in the diet. Recent experimental evidence indicates that the presence in the diets of certain vitamins (notably thiamin, riboflavin and nicotinic acid), minerals (phosphorus, magnesium, manganese, zinc) and possibly other catalytic or enzymatic agents, play a more fundamental role than the relative proportions of the energy-bearing foodstuffs (fat, protein and carbohydrate). Although these new findings may seem more pertinent at the moment, it is prudent not to omit, discard nor minimize the more familiar considerations, in view of equally significant implications presented by studies of specific amino acids and fat constituents in the synergism of energy and general metabolism.

Fat Energy

Fat is the richest dietary source of energy, yielding more Calories per unit weight than any other food principle, and plays an important role in the energy metabolism of the body. It is the conveyor of unsaturated fatty acids, fat-soluble vitamins and other organic substances which are essential aids in metabolism in general and of proteins and energy in particular. Lipids vary in composition, from the simple forms composed of esters of fatty acids and glycerol, to the complex varieties containing additional groups of phosphoric acid and nitrogen (lecithin, cephalin and sphingomyelin), or carbohydrate and nitrogen (cerebrosides), and in some cases, amino- and sulfo-groupings. During digestion the lipids are split into their component parts, each of which may have a distinctive physiological role. The glycerol and fatty acid fractions of fat molecules participate most actively in the energy phase of metabolism.

Satisfactory fat oxidation (beyond the four-carbon stage) can take place only when some carbohydrate can be metabolized. Under normal dietary conditions there is a sufficient excess of carbohydrate to permit complete oxidation of the fatty acids to carbon dioxide and water; however, if there is an insufficient amount, incomplete oxidation of the fatty acids occurs and the unoxidized products, such as ketone bodies, beta-oxybutyric acid, diacetic acid and acetone appear in the blood and are excreted in the urine. (See p. 91.)

Evans and Lepkovsky[87, 88, 89] were among the first investigators to demonstrate that the liberal inclusion of fat in the diet results in a sparing of thiamin. There was convincing evidence introduced several years ago that suggests that thiamin plays a role in the synthesis of fat from carbohydrate, in the animal body[216,377,378] and since that time an active interest in the various essential metabolic constituents for the synthesis of fat in the animal body is evidenced by the many relevant scientific communications in the literature.

Protein Energy

Protein taken into the body first undergoes digestive hydrolysis, then absorption, after which the nitrogen end products are only partially combusted. Of these partially combusted nitrogenous end products of metabolism, some are excreted in the urine, others may be synthesized into glycogen or body proteins, depending upon the availability of foodstuffs in the diet and the current needs of the organism.[206] Under usual conditions of protein intake, even in healthy growing children, much of the protein ingested is used to provide energy. The amount of nitrogen retained may be influenced by the total number of calories consumed and by the amounts of these furnished as fat and carbohydrate, for both have a protein-sparing effect.[206] The relation of the average daily energy intake to the accompanying mean daily protein retentions is illustrated in Table 48.

TABLE 48

RELATION OF AVERAGE DAILY PROTEIN RETENTION TO AVAILABLE ENERGY* IN 18 CHILDREN 4 TO 12 YEARS IN AGE, DURING 2515 EXPERIMENTAL DAYS†

Age group	Available energy*		Protein intake		Protein retention‡	
year	Cal./day	Cal./kg.	gm./day	gm./kg.	gm./day	gm./kg.
4	1679	93	62.5	3.5	3.9	.21
5	1582	85	61.4	3.3	3.5	.19
6	1723	79	63.8	2.9	2.6	.12
8	2010	77	74.6	2.8	3.1	.12
9	1975	69	81.6	2.8	6.6	.23
10	2301	70	82.9	2.5	5.9	.18
11	2297	64	82.9	2.3	5.1	.14
12	2289	55	82.9	2.0	5.0	.12

* Physiologic fuel value (net Calories).

† See Table 24, page 92 for body weights and lengths of subjects, distribution among age groups and number of balance periods for each group.

‡ Difference between nitrogen intake and outgo (urine+feces)×6.25.

In order to meet the increasing energy and protein needs of childhood it is necessary to provide increased dietaries for older children. The diet of the 12 year old boy provided 610 Calories and 20 gm. of protein more than did the diet of the four year old child (Table 48), although the larger intake for the older child contributed fewer Calories and less protein per kilogram of body weight. Assuming that the nitrogen retained was held as protein, then an average of approximately 4 gm. of protein was acquired daily by each child. In this study the diet furnished sufficient energy, vitamins, minerals and protein of good quality so that the bodies could select what they needed. Very different results might be obtained with children of a different nutritive background or on restricted diets.

Carbon and Nitrogen Ratio: The carbon and nitrogen contents of the urine, together with the caloric value, give significant information on the physiological fuel value of protein and fat. The nitrogenous and carbonaceous compounds (urea, uric acid, creatinine, creatine) of the urine are the normal oxidation end products of protein metabolism. For these reasons the carbon to nitrogen ratio of the urine is important in considering energy metabolism, particularly in abnormal states. Every gram of urinary nitrogen eliminated is accompanied by 0.6 to 0.9 gm. of carbon and represents the disruption of the amino acids of approximately 6.25 gm. of protein. The greater the excretion of incompletely oxidized carbonaceous and nitrogenous constituents the greater the latent heat loss from the body.

The average potential energy lost in urine and feces amounted to 92 and 105 calories per 24 hours, respectively, for seven children, ages 8 to 12 years. Of the average daily intake of carbon (206 ± 12 gm.) 8.6 ± 0.3 gm. were excreted in the urine and 9.5 ± 1.1 gm. in the feces. Four and two-tenths (4.2) per cent of the carbon intake was excreted by way of the kidneys, 4.6 per cent in the feces, leaving 91.2 per cent available for body use, which corresponds to the 92 per cent of the gross energy of the diets which was available for use by the body. With an average daily urinary nitrogen excretion of 11.0 gm. the carbon to nitrogen ratio was 0.78.

From 17 to 21 per cent of the dry weight of urine and 40 to 47 per cent of the dry weight of the feces is carbon. The average energy values per gram of dry urine and per gram of urinary nitrogen are 2.0 and 8.4 Calories, respectively, and per gram dry feces and per gram fecal nitrogen, 5.4 and 81 Calories, respectively. These values for the carbon, nitrogen and energy of urine and feces of healthy children, obtained under highly standardized methods of collection, sampling and preservation of specimen, should be of service for comparative purposes in the study of certain diseases of childhood in which there occur disturbances in energy, protein or fat metabolism. The carbon to nitrogen ratio, as an indicator of the completeness of oxidation of the urinary constituents, has been demonstrated to give helpful and suggestive information on the role of the vitamin B fractions in metabolism.[187]

Nitrogen: Sulfur Ratio in Urine: Almost all of the sulfur compounds found in the urine are the result of protein metabolism. If there is not sufficient energy in the diet to take care of the current activity and growth needs, an excessive amount of protein will be used as fuel, thus causing increased excretion of both urinary nitrogen and sulfur. Because they are involved in related metabolic processes the ratio in urine, of nitrogen to sulfur has often been considered of interest, although its importance and interpretation are questionable and are largely a reflection of the N:S ratio of the diet. The ratios of nitrogen to sulfur in the urine of the children are given in Table 49. The children of this study were provided with approximately 26 to 47 per cent of energy in excess of the basal heat production. As the nitrogen intake was increased with age both the urinary nitrogen and sulfur were augmented. The correlation coefficients between nitrogen intake (503 cases) and the urinary sulfur and nitrogen excretions were very high, 0.90 and 0.92, respectively.

TABLE 49

NITROGEN:SULFUR RELATIONSHIPS IN AVERAGE DAILY URINARY EXCRETION OF 13 BOYS DURING 1635 EXPERIMENTAL DAYS

Age group	Boys	Periods	Nitrogen intake	Fuel value of intake*			Urinary		
				CHO	Fat	Protein	Nitrogen	Sulfur	N/S
year	number		gm.	per cent	per cent	per cent	gm.	gm.	
4	1	43	10.00	49	38	15	8.32	0.570	14.6
5	1	41	10.04	50	39	15	8.24	0.532	15.5
6	2	90	10.30	48	39	14	8.85	0.595	14.9
8	4	98	11.93	48	41	15	10.29	0.691	14.9
9	1	11	13.06	51	38	16	10.77	0.742	14.5
10	2	22	13.27	45	44	14	11.14	0.775	14.4
11	1	11	13.27	45	44	14	11.05	0.772	14.3
12	1	11	13.27	46	44	14	11.04	0.760	14.5

* Physiologic fuel value.

Whether or not overfeeding occurred in this group of children was not determined. If overfeeding did exist at any time it would be interesting to know to what extent it influenced the urinary nitrogen, and urinary sulfur excretion and the degree of nitrogen retention.[58,59,60] Not only is the total energy intake highly significant in protein metabolism but the proportion of carbohydrate and fat in the diet is important. In the diets of 13 boys approximately 86 per cent of the energy was represented as fats and carbohydrates, the remainder of about 14 per cent were of protein origin (Table 49). Under the conditions of the dietary imposed by our metabolic procedures the total energy consumption was undoubtedly a factor influencing protein metabolism. The high correlation coefficients of urinary nitrogen and sulfur to caloric intake (0.85 ± 0.01 and 0.81 ± 0.02) in 488 cases may be a considerable extent the result of having held the proportion of protein Calories in the diet constant.

Creatinine Coefficient*

Folin's early studies of the laws governing the chemical composition of urine showed that the output of creatinine of men, upon a meat-free diet is "a constant quantity, different for different individuals, but wholly independent of quantitative changes in the total amount of nitrogen eliminated."[104] Recent investigators find, however, that the creatinine output is not so constant as Folin indicated.[149] The variations in the daily output of creatinine by two of our subjects is shown by the creatinine coefficients over a period of 55 consecutive days (Figure 45). Shaffer[301] showed that the rate of creatinine elimination is just as uniform from hour to hour as it is from day to day. While

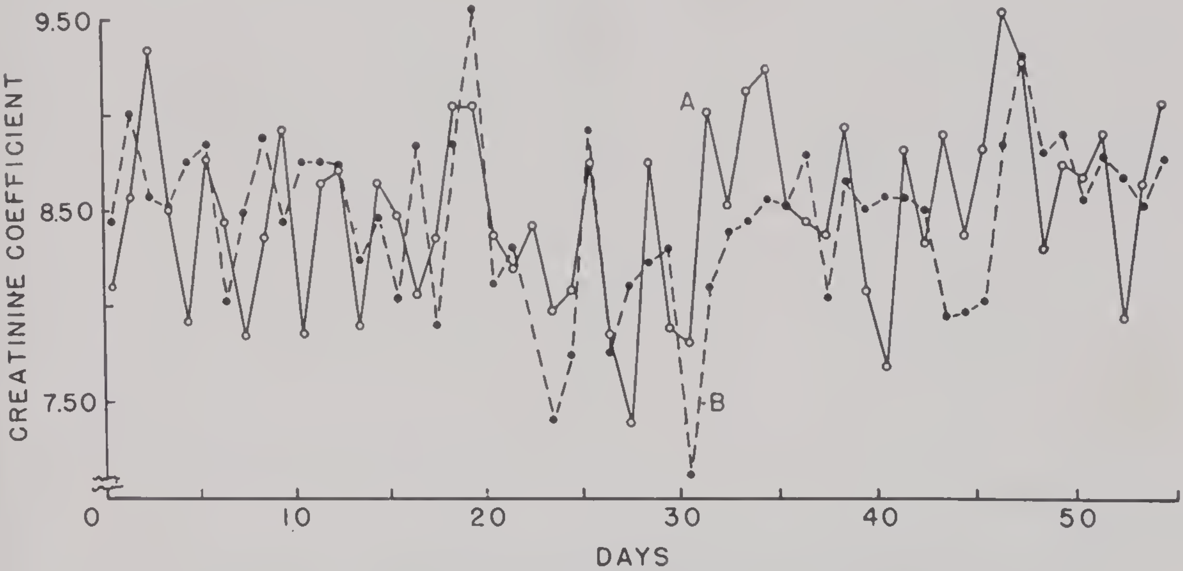

FIGURE 45. Creatinine coefficients of two children (A, B) during 55 consecutive days.

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* In determining creatinine coefficients we have used milligrams of *preformed* creatinine nitrogen per kilogram of body weight. (Creatine was not included. See method, p. 282)

the daily excretion of creatinine is relatively constant for the individual, the variations among subjects were considered largely dependent upon simple differences in body weight. Folin showed that endogenous protein metabolism which resulted in the constant, even production of creatinine is a manifestation of cell metabolism, while Shaffer believed that the creatinine excretion was an index of muscular development.[161]

TABLE 50

BASAL AND CREATININE METABOLISM IN RELATION TO WEIGHT

	Subjects		Basal heat production			Preformed creatinine		
	Weight*	Number	Tests	Mean	Range	Days	Mean	Range
	kg.		number	Cal./24 hours		number	mg./24 hours	
Boys	42–	1	2	1463	90	10	936	0
	40–	1	2	1410	215	10	974	0
	34–	2	6	1347	69	30	890	57
	32–	2	4	1386	65	20	784	186
	30–	1	2	1308	6	10	681	0
	28–	2	12	1181	554	60	672	172
	26–	1	4	1233	111	20	639	18
	24–	2	9	1040	183	45	618	204
	22–	2	9	1018	151	45	520	81
	20–	1	4	994	67	20	527	54
	18–	2	16	988	168	80	426	72
Girls	28–	1	2	1274	61	10	613	0
	26–	1	2	1344	79	10	581	0
	24–	1	5	1015	228	25	505	22
	22–	2	11	978	247	55	483	61
	20–	1	6	972	88	30	463	89
	18–	1	7	853	57	35	418	111

* Dashes indicate that the values given represent the beginning of the class interval rather than the midpoint.

There is an increase in both the absolute and relative excretion of creatinine from birth to puberty, the rate of increase being greatest during the initial months of life. The creatinine coefficient has been variously interpreted[141] as proportional to, or an index of (1) the amount of active protoplasmic tissue in the body, by Folin; (2) the muscular mass and efficiency of the individual, by Shaffer. We have analyzed samples of daily urine composites which have been collected and preserved under carefully controlled conditions and the analyses made immediately after collection of the composites was completed. From these values we have found that children ranging in age from 4 to 12 years exhibit a preformed creatinine coefficient of 7.4 to 9.7, parallel with age. These data indicate that the preformed creatinine output of growing children rises, concomitant with increase in body weight, in such a way that young children may show a creatinine coefficient comparable with that established as the normal adult standard, i.e., the adult level (7 to 11) is reached much earlier than has been supposed.

Creatinine Excretion versus Basal Heat Production: Palmer, Means and Gamble[260] suggested, later it was proved by Talbot,[340] and others, that urinary creatinine excretion is directly related to basal metabolism in children. In our normal children the relationships between daily basal heat production and preformed creatinine and between basal heat production and body weight were similar (correlation coefficients were 0.76 ± 0.04 and 0.79 ± 0.02, respectively) and the data demonstrated a close relationship (Figure 46) between surface area of the body and the creatinine nitrogen

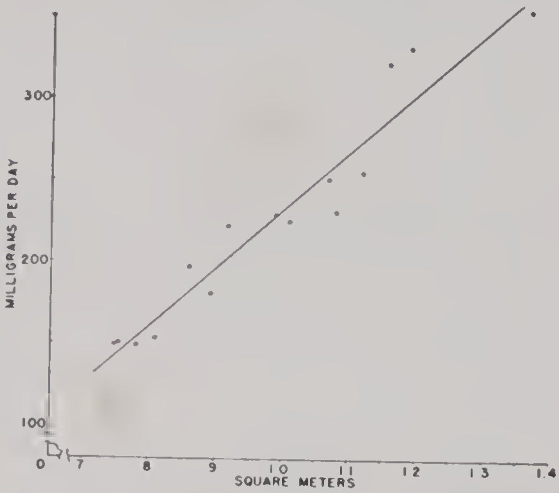

Figure 46. Body surface area *vs.* creatinine nitrogen output of children. Each point represents the daily average for one child.

TABLE 51

BASAL AND CREATININE METABOLISM IN RELATION TO LENGTH

	Subjects		Basal heat production			Preformed creatinine		
	Recumbent length*	Number	Tests	Mean	Range	Days	Mean	Range
	cm.		number	Cal./24 hours		number	mg./24 hours	
Boys	155	1	4	1436	215	20	955	38
	145-	1	2	1368	22	10	915	0
	140	2	6	1436	172	30	706	285
	135-	3	16	1191	439	80	740	250
	130-	1	4	1233	111	20	639	18
	120-	3	22	1022	151	110	561	234
	115-	1	8	992	122	40	428	83
	110	1	7	979	164	35	426	51
	105	1	1	1013	0	5	398	0
Girls	130-	1	4	1309	141	20	597	32
	120-	1	6	1004	228	30	500	35
	115-	2	10	980	247	50	484	61
	110-	1	6	972	88	30	463	89
	105	1	7	853	57	35	418	111

* Dashes indicate that the values given represent the beginning of the class interval rather than the midpoint.

output. Table 50 shows the average preformed creatinine excreted per 24 hours, by 24 children during 103 balance periods of five days each. Basal metabolism tests were made during each period.

Investigators have shown a direct relationship between height and preformed creatinine excretion. Table 51 shows this relationship for subjects of our studies.

Figure 47 shows the relation of the creatinine excretion to basal caloric output per 24 hours.

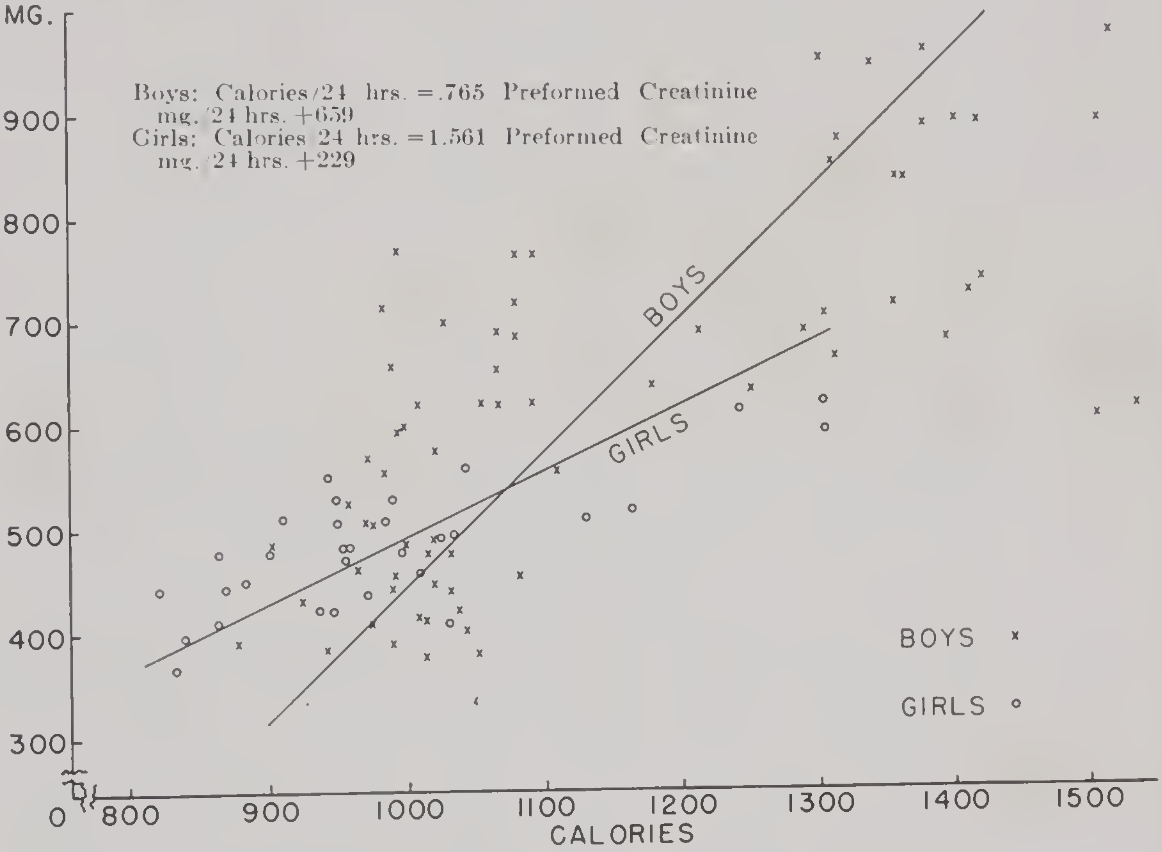

Figure 47. Creatinine excretion *vs.* basal heat production per 24 hours. According to the regression equations shown in the figure, the basal Calories per day corresponding to various levels of daily creatinine excretion are:

Creatinine (mg.)	400	450	500	550	600	650	700	750	800	850	900	950
Basal Calories												
Boys:	965	1003	1042	1080	1118	1156	1194	1233	1271	1309	1348	1386
Girls:	853	931	1010	1088	1166	1244	1322	1400	1478	1556	1634	1712

Talbot, Worcester and Stewart[341] recently have presented new creatinine standards for basal metabolism and clinical application for children. Their data show that the creatinine standard is as accurate as weight standards for children who are normal, and suggest that it is of greater accuracy for those who are abnormal, especially the obese, because creatinine excretion is an index of muscle weight.

Neutral Sulfur

In a study of sulfur excretion, Folin[104] discovered that the neutral sulfur excretion tends to be relatively constant and independent of a normal protein intake. According to Amann and Mourat[39] the factors affecting the metabolism of energy also affect the excretion of neutral sulfur, but the neutral sulfur is not definitely proportional to calorie production. They point out that neutral sulfur excretion is the same on low as on "normal" protein diets but is increased on high-protein diets. When the dietary protein is increased 15 times, neutral sulfur excretion is tripled. In other words, the amount of neutral sulfur is not strictly endogenous, originating from destruction of tissue protein only. It is not altogether independent of the diet, although, of course, it is relatively constant, i.e., in comparison with total sulfur excretion. The variability of neutral sulfur excretion, and its relation to the variations in energy and creatinine has been reviewed by Brody.[39] The average daily neutral sulfur excretion of seven children, 8 to 12 years old, during 45 consecutive days, ranged from 60 to 82 mg. with a mean of 73 ± 14 mg. The neutral sulfur accounted for 8 to 11 per cent of the urinary sulfur excretion.

Adequacy of Energy and Protein Intakes

During high levels of protein feeding the body may be very wasteful of nitrogen, even during the augmented needs of growth; however, restricted nitrogen consumption may lead to nitrogen economy. By our group of well-nourished and well-fed subjects, only five to eight per cent of the nitrogen consumed was retained. Over a number of balance periods all children showed nitrogen retentions but during short intervals (5 to 15 days) individuals occasionally showed a loss of nitrogen from the body for no discernible reason. Because of the unaccountable fluctuations in nitrogen retention we have tested, insofar as possible, the adequacy of the protein and total energy intakes.

From the data obtained on the energy exchange of children we may calculate whether sufficient calories were allowed to meet the current energy needs of the body and provide a reasonable excess for activity and growth. Although the potential energy value of the daily diets of 13 boys ranged from 1800 to 2500 Calories, from 130 to 210 Calories were lost through the urine and feces, leaving the energy value 1650 to 2300 Calories (Table

52*A*), or an average 79 Calories per kilogram of body weight per day. The basal heat production averaged from 980 to 1460 Calories per 24 hours, which represented 56 per cent of the net Calories of the diets. Assuming that the specific dynamic action of foods is represented by six per cent of the net energy intake (DuBois[68]), after deducting the 99 to 138 Calories lost in this manner and that lost as basal heat, there still remained on an average about 711 Calories or 38 per cent of the net energy which was available for work. This would seem to be a conservative allowance for healthy active children. If we estimate the amount of protein metabolized as energy by multiplying the urinary nitrogen excreted per 24 hours by 26.51* in accordance with Lusk's recommendation, we find that this group of children metabolized from 220 to 295 Calories in the form of protein.

TABLE 52*A*

ESTIMATED AVERAGE DAILY ENERGY EXCHANGE OF 13 BOYS DURING 1635 EXPERIMENTAL DAYS

Values per 24 hours

	AGE GROUPS OF CHILDREN								
	4	5	6	8	9	10	11	12	Average
Children in group	1	1	2	4	1	2	1	1	
Five-day balance periods (number)	43	41	90	98	11	22	11	11	327
Potential food energy* (Cal.)	1813	1818	1921	2178	2187	2495	2499	2499	2058
Potential heat lost † (Cal.)	134	161	144	168	207	194	202	210	162
in urine (Cal.)	63	66	68	80	88	94	89	99	75
in feces (Cal.)	71	95	76	88	119	100	113	111	87
Physiologic fuel value‡ (Cal.)	1679	1657	1777	2010	1980	2301	2297	2289	1896
per kg. body weight (Cal.)	93	86	81	77	68	70	64	55	79
per sq. m. surface area (Cal.)	2288	2095	2063	2055	1831	2008	1970	1659	2065
Basal Heat Production (Cal.)	983	978	1010	1072	1462	1358	1336	1436	1071
per kg. body weight (Cal.)	54	51	46	40	50	41	37	35	45
of net Calories (per cent)	58	59	57	53	74	59	58	63	56
Specific dynamic action of food § (Cal.)	101	99	107	121	119	138	138	137	114
ENERGY AVAILABLE FOR ACTIVITY# (CAL.)	595	580	660	817	399	805	823	716	711
OF PHYSIOLOGIC FUEL VALUE (%)	35	35	37	41	20	35	36	31	38

* Gross Calories determined by heat of combustion of food.
† Heat of combustion of urine and feces.
‡ Heat of combustion of food minus heat lost through urine and feces.
§ Six per cent of the net or physiologically available energy.[68]
# Fuel value minus basal heat production and specific dynamic action of food.

---

* One gm. urinary nitrogen represents 26.51 Calories derived from protein.[206]

The relation between the basal metabolism and the endogenous nitrogen metabolism to protein requirement has been studied in this country by Mitchell and associates.[319] It has been demonstrated that the maintenance requirement for protein parallels surface area more closely than it does body weight.[319,352] Since Sorg-Matter[323] found that age did not disturb the relationship when the plane of nutrition was unaltered,[352] it seems justifiable to calculate endogenous nitrogen from basal heat. The endogenous nitrogen output has been found to be proportional to the surface area and is so related to the basal metabolism that 2 mg. urinary nitrogen per day were lost per Calorie of basal heat produced. Assuming that each Calorie of basal heat lost is equivalent to 2 mg. of endogenous nitrogen excreted in the urine (2×6.25 = 12.5 mg. of protein), the average daily endogenous protein loss ranged from 12 to 18 gm. per day for children between the ages of 4 to 12 years with an average value of 13 gm. of protein (Table 52*B*).

TABLE 52*B*

ESTIMATED AVERAGE DAILY PROTEIN SURPLUS OF 13 BOYS DURING 1635 EXPERIMENTAL DAYS

Values per 24 hours

	AGE GROUPS OF CHILDREN								
	4	5	6	8	9	10	11	12	Average
Children in group	1	1	2	4	1	2	1	1	
Five-day balance periods (number)	43	41	90	98	11	22	11	11	327
Protein intake (gm.)	62	63	64	74	82	83	83	83	70
per kg. body weight (gm.)	3.5	3.3	2.9	2.8	2.8	2.5	2.3	2.0	2.9
per sq. m. surface area (gm.)	85.2	79.3	74.7	76.1	75.1	72.1	70.8	59.9	76.3
Urinary nitrogen (gm.)	8.3	8.2	8.8	10.3	10.8	11.1	11.0	11.0	9.5
Available protein energy* (Cal.)	248	252	256	296	328	332	332	332	280
Protein metabolized as energy† (Cal.)	220	218	234	273	285	295	293	292	252
Protein absorbed (gm.)	56	55	58	68	72	76	74	74	63
Endogenous protein loss‡ (gm.)	12	12	13	13	18	17	17	18	13
Estimated endogenous protein requirement§	24	24	26	26	36	34	34	36	27
SURPLUS PROTEIN AVAILABLE✚	32	31	32	42	36	42	40	38	36

* Protein in grams times 4.

† Urinary nitrogen times 26.51.[206]

‡ Endogenous urinary nitrogen output bears a ratio to the basal heat production of approximately 2 mg. nitrogen, or 12.5 mg. conventional protein (i.e., 2×6.25) to the Calorie. The protein (mg.) actually utilized in the replacement of endogenous losses may be estimated by multiplying the basal heat production by 12.5.[319]

§ Assuming a biological value of 50 per cent for dietary protein utilization the endogenous loss may be doubled.[319]

✚ Protein absorbed minus estimated endogenous protein loss.

In this connection Smuts[319] states that "the protein requirement for maintenance refers not to dietary protein, nor to digestible protein, but to protein actually utilized in the replacement of endogenous losses. The corresponding amount of dietary protein would be larger than this, and would be greater, the greater its wastage in metabolism and hence the smaller its biological value." Therefore, a safe allowance of dietary protein to cover endogenous requirement may be estimated by doubling the calculated endogenous protein loss. Using this procedure, the average daily endogenous protein requirement of 13 boys during 1635 days amounted to 24 to 36 gm. (average 26 gm.) under the specific dietary and experimental regime employed. This assumes that in the function of maintenance the biological value of the dietary protein was only 50 per cent, or less than average quality. As a matter of fact the actual coefficient of digestibility of the protein of the diet was 80 to 90 per cent.

The average daily total protein consumption, largely derived from animal sources and therefore of high quality, was augmented from 62 to 83 gm. as age progressed, although the quantity of protein per kilogram of body weight per day decreased from 3.5 to 2.0 gm. Of the 62 to 83 gm. of protein consumed, from 55 to 76 gm. were absorbed. On the basis of the above calculations, the children had a daily surplus of 31 to 42 gm. (average 37 gm.) of absorbed protein available over and above the estimated daily endogenous requirement. Moreover, the dietary carried a surplus of non-protein energy, 240 to 268 Calories, in excess of the basal requirement (Table 52*A*), which could function in sparing protein. In addition, the diets included adequate supplies of vitamins and manganese, zinc, copper and iron, which promote the catalytic processes in food utilization. In view of these facts it seems unlikely that those amino acid materials which serve in the functions of growth and maintenance would ever be needed as sources of energy. Other evidence of the adequacy of the protein intakes of our subjects is presented in the discussion of nitrogen balances. (See p. 158).

CHAPTER VIII

METABOLIC BALANCES

THE utilization of energy and dietary components is directly dependent upon intake, at least to the extent that an organism cannot accumulate the optimal quantities of food constituents or expend the proper amount of energy unless the materials are made available for use. The "optimal" intake of a food component has been assumed to be the amount which produces the highest retention, although recent evidence has cast doubt upon whether the *highest* retention is necessarily the most *desirable* retention.

Erroneous concepts may be gathered by studies involving only one (or a few) of the substances used by the body from a particular dietary, for assimilation of individual nutritive substances has been shown to be dependent upon qualitative characteristics of the dietary as well as quantitative considerations. The influences of proportionality in diets upon retention go beyond the effects of relationships in the quantities of fat, protein, minerals, and carbohydrate in the food intake. The distribution of the essential and non-essential amino acids composing the protein and of available and complex (fiber) carbohydrates influence the physiologic activities of the body. The presence of vitamins and "heavy metals" is essential for adequate utilization of food substance.

The value of metabolic balance results is directly proportional to the length of the investigation and each metabolic balance period should be of sufficient length to provide facility in the conduct of the study but should not be long enough to conceal variation in physiologic response (p. 36). In interpreting the results obtained by balance techniques, many additional considerations confront the research worker or the clinician. In earlier chapters we have stressed selection, standardization and control of experimental subjects and environment; evaluation of dietary requirements and controlled sources of food supply; and, accurate methods of sampling food intakes and collecting the outgo (urine and feces) from each period of intake. Equally exact procedures must be applied in the laboratory; in preparing material for analysis, in making the analyses, and in recording the results of all determinations.

Prodigious efforts expended in accomplishing with maximum efficiency all of the foregoing, may be minimized, or even wasted, if the results are invalidated by misinterpretation or unjustified manipulation of the data. After the analytical values have been obtained, the data must be compiled before it is fully usable for interpretation. Results from physiological and biochemical studies provide opportunity for calculation of the data in several different ways and upon several bases. The selection of mathematical treatment, to a large extent, is determined by the type and number of data procured and the basis upon which interpretation is to be made. If interpretation upon several bases is justified by the character of the data, each possibility should be explored. The methods of recording, recapitulating and analyzing the data obtained in our investigations of normal children are detailed in Chapter X (p. 364).

Chronological age is intimately involved with the accretion of chemical materials by the bodies of infants and children. It is obviously true that a twelve year old child must assimilate larger amounts of food nutrients per day than are required by a six-year-old; yet the younger child may be accumulating new body substance at a greater rate, in proportion to his size, than is the older child. Children of the same chronological age exhibit wide variability in their assimilation of materials from food, and evidence has been presented which indicates that chemical growth may be more closely related to physiological than to chronological age. The relationship of chemical growth to physiological age involves minute analysis of comprehensive information upon individual children and has been reserved for the second volume of this presentation. Data presented in this Chapter have been given as averages for chronological age groups, not to imply a conformity between our results and age, but to enable the reader to compare the various mean responses at different stages in childhood (4 to 12 years).

NITROGEN

In the past, estimations of nitrogen requirement have been concerned, primarily, with protein requirement. Greater knowledge of the composition of different proteins and their nutritive behavior in animal experiments have shown that the principal value of a protein resides in its component parts, the amino acids.[238,283] Proteins may contain twenty or more amino acids and the array of proteins in body tissues and fluids contain a great variety of combinations of these protein "building stones." Some of the individual amino acids can be synthesized within the body, others must be furnished in food. The amino acids which cannot be synthesized by the body and are necessary for growth or maintenance are known as essential amino acids. The tentative list of essential amino acids established by rat experiments, contains the names lysine, tryptophane, histidine, phenylalanine, leucine, isoleucine, threonine, methionine, arginine and valine. Examples of some of the known synergistic relationships which exist, between the amino acids which are not absolute essentials and those which are indispensable, have been discussed in connection with food supply (pp. 88–90). In rats, approximately 50 per cent of the nitrogen requirement for body maintenance demands specific amino acid structures, while the remainder may be satisfied by a variety of incomplete amino acid mixtures. That other food elements such as vitamin C are essential to the normal utilization of amino acids has been demonstrated recently.[196,299]

Studies with rats suggest the significance of specific amino acids in the maintenance and growth of all animals, however, the conclusions derived cannot be applied directly to nutrition problems of man. Specific information is not available upon the daily intake of, or requirement for amino acids by human subjects,[10] although some efforts in this direction are being made.[1,145]

Tables 53–56 contain the average daily nitrogen balances of 20 boys and nine girls, during 2,965 experimental days. The mean average daily nitrogen retention was 0.62 gm. If this amount of nitrogen were added to a body as protein (nitrogen ×6.25) it would represent a daily accretion of 3.9 gm. protein, or approximately 20 gm. of body substance, according to Rubner's estimate of one gram of nitrogen being equivalent to 33 gm. body substance composed largely of muscle. An average body weight gain of about one-half this amount (11.7 *vs.* 20 gm.) was observed.

This indicates some discrepancy between our data and Rubner's value. However, the fact should not be overlooked that composition of weight gain differs in quality; sometimes it may consist in large measure of fluids, and at other times to a greater extent of fat or active protoplasmic muscle tissue. The possibilities of these interpretations will be expanded in Volume II.

The variability of nitrogen retention in childhood has been pointed out.[220] A wide range of variation is demonstrated by the large standard deviations for the average retentions of each age group. The correlation coefficient of nitrogen retention with age was 0.20 ± 0.04. There are, apparently, numerous controlling factors operating to affect the rapidity of nitrogen storage. Some of these factors do not operate continuously and therefore the organism seems to oscillate in its nitrogen needs. The retention of nitrogen was only slightly influenced by body weight, having a correlation coefficient of 0.22 ± 0.4; even less relationship to surface area was indicated by the correlation coefficient 0.16 ± 0.04.

TABLE 53

AVERAGES OF 593 AVERAGE DAILY NITROGEN BALANCES FOR 29 CHILDREN*

Values in grams

Age group	Intake		Outgo				Retention‡			Absorption§
			Urine		Feces					
year	mean	SD†	mean	SD†	mean	SD†	mean	SD†	per cent	mean
4	9.58	.77	7.81	.92	1.07	.13	0.70	.47	7	8.51
5	9.74	.76	8.05	.73	1.08	.17	0.61	.39	6	8.66
6	10.17	.59	8.65	.56	1.06	.22	0.46	.51	5	9.11
8	11.56	1.35	9.81	1.45	1.13	.17	0.62	.56	5	10.43
9	13.06	.41	10.62	.30	1.39	.22	1.05	.45	8	11.67
10	13.04	1.30	10.78	.88	1.23	.21	1.03	.86	8	11.81
11	13.27	.42	11.04	.27	1.42	.10	0.81	.38	6	11.85
12	13.27	.42	11.04	.37	1.43	.17	0.80	.27	6	11.84

* In Table 20, page 87, are shown the distribution of the subjects among the age groups; the distribution of the five-day balance periods among the subjects; and, the average body weights and recumbent lengths of the subjects in each age group during the balance periods of study.

† The standard deviation (SD) was obtained with the formula: $\sqrt{\frac{\Sigma x^2 - N\bar{x}^2}{N-1}}$ in which N is the number of values, Σx^2 is the sum of the squares of the values, and $\bar{x}^2$ is the square of the mean of all the values.

‡ Retention is calculated as "Intake minus Outgo."

§ Absorption is calculated as "Intake minus Feces."

Considerable stress has been placed upon the interpretation of metabolic data on the basis of body weight, particularly data involving the elements which are intimately related to the soft tissues. Relation of nitrogen intake to retention upon a basis of milligrams of nitrogen per kilogram of body weight per day shows little or only slight relationship for the subjects of our studies (correlation coefficient, 0.31 ± 0.04. The average daily nitrogen retention was approximately 27 mg. per kilogram of body weight and the values varied within a broad range, shown by a standard deviation of 22.

TABLE 54

DISTRIBUTION OF NITROGEN EXCRETION BETWEEN URINE AND FECES IN 593 NITROGEN BALANCES FOR 29 CHILDREN

Age group	EXCRETION OF NITROGEN INTAKE		TOTAL DAILY EXCRETION IN URINE AND FECES		
	Urine	Feces	Mean	Urine	Feces
year	per cent	per cent	mg.	per cent	per cent
4	82	11	8.88	88	12
5	83	11	9.13	88	12
6	85	10	9.71	89	11
8	85	10	10.94	90	10
9	81	11	12.01	88	12
10	83	9	12.01	90	10
11	83	11	12.46	89	11
12	83	11	12.47	89	11

The interpretation of nitrogen balance data on the unit of body weight basis would seem to indicate that the metabolic

TABLE 55

AVERAGES OF 593 AVERAGE DAILY NITROGEN BALANCES FOR 29 CHILDREN

Values in milligrams per kilogram of body weight

Age group	Intake		Outgo				Retention		Absorption
			Urine		Feces				
year	mean	SD	mean	SD	mean	SD	mean	SD	mean
4	522.1	59.0	426.9	67.7	58.1	8.4	37.1	24.4	464.0
5	520.1	34.6	429.9	40.3	57.8	7.7	32.4	20.2	462.3
6	466.6	25.8	397.3	34.7	48.9	8.8	20.4	22.7	417.7
8	442.9	52.3	375.3	52.8	43.3	7.2	24.3	22.4	399.6
9	457.3	16.2	371.6	11.0	48.7	6.9	37.0	16.2	408.6
10	393.2	41.6	325.1	29.8	37.0	5.5	31.1	25.1	356.2
11	371.3	6.4	309.1	6.4	39.7	3.0	22.5	10.8	331.6
12	317.1	4.0	263.9	7.1	34.2	3.8	19.0	6.8	282.9

technique of feeding a generous supply of protein in considerable excess of the actual tissue needs and permitting the body to choose what nitrogen it needs, has an advantage over the laborious method of adjusting the feeding of protein on the basis of per kilogram of body weight per day. Our children received a liberal supply of dietary nitrogen, ranging from 522 mg. for the four-year-old and decreasing to 317 mg. for the twelve-year-old, an average daily consumption of 473 mg. per kilogram of body weight per day for all the children.

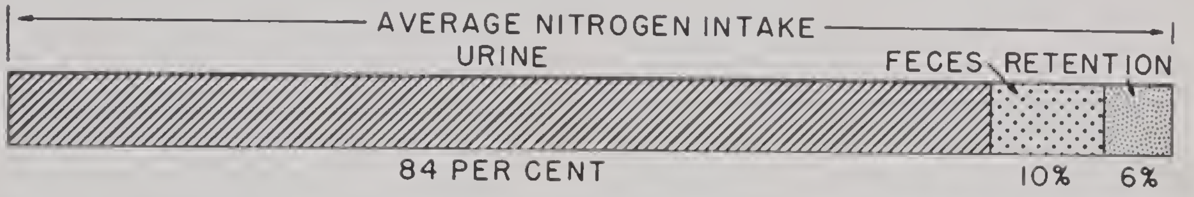

Figure 48. Average retention and excretion of nitrogen intakes.

The quantity, quality, digestibility and availability of the protein consumed have important bearing upon the amount of nitrogen retained by the body. The nitrogen eliminated in urine represents the end products of a chain of physiological events which includes digestion, absorption, intermediary metabolism and excretion. Some of the fecal nitrogen may arise from similar metabolic sources, but, in addition, the feces contain nitrogenous products of other origin; undigested food residues, gastrointestinal secretions or excretions, bacteria and their by-products. No analytical method is known by which the fecal nitrogen which has been utilized by the body (metabolic origin) can be distinguished from the portions which have not been assimilated. Urinary nitrogen, which represents more than three-quarters of the food nitrogen and approximately 90 per cent of the total excretion of nitrogen from the child's body, has entered into the body economy, while the greater part of the 10 per cent found in the stools probably has not been of biological value to the child.

Nitrogen metabolism data have greater significance when considered in relation to nitrogen intake. The average distribution of the nitrogen intakes given in Table 53 is shown in Figure 48. During the 593 balance periods, nitrogen retentions ranged from five to eight per cent of the intakes. Nine to 11 per cent of the nitrogen intake was found in the feces and 81 to 85 per cent in the urine. The full physiological role of food protein is not disclosed by interpretations based upon nitrogen retention values or the percentage distribution of the nitrogen intake between the urine, feces and retention. In interpreting investigations of nitrogen metabolism, various formulas have been advocated in attempts to determine a true measure of nitrogen assimilation. The results of calculations according to these formulas have been assigned various designations but the true interpretation of the figure has not always been clear. Values for nitrogen "absorption" are especially pertinent in an attempt to estimate nitrogen utilization.*

TABLE 56
AVERAGE DAILY RETENTION AND ABSORPTION OF NITROGEN

Nitrogen intake	Children	5-day balances determined	Initial		Nitrogen absorption	Nitrogen retention	
			Age	Weight			
gm.	number	number	months	kg.	per cent intake*	per cent intake	per cent absorption†
8	6	23	67	20.5	88	13	15
9	4	83	70	18.2	89	8	9
10	9	319	72	19.5	89	5	6
11	1	45	99	27.8	90	2	3
12	2	46	102	25.2	90	7	8
13	7	77	124	32.3	90	7	7

* $\frac{\text{Absorbed Nitrogen}}{\text{Food Nitrogen}} \times 100$ (sometimes called coefficient of protein digestibility).

† $\frac{\text{Retained Nitrogen}}{\text{Absorbed Nitrogen}} \times 100$ (sometimes called apparent utilization).

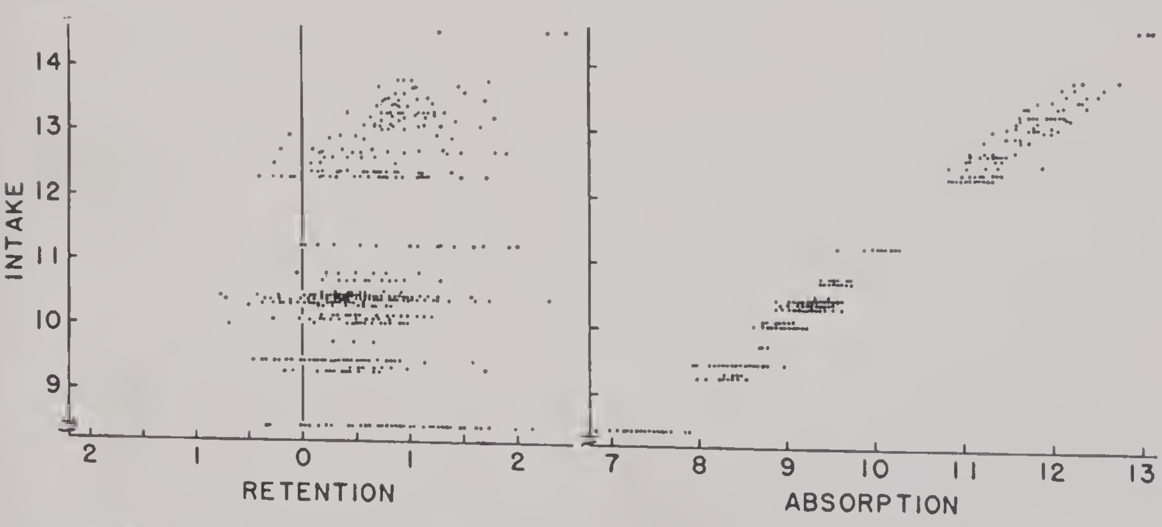

FIGURE 49. Relationship between retention and absorption of nitrogen intake.

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* The arbitrary meanings assigned to the terms *retention*, and *absorption* should be considered in connection with the discussion of nitrogen metabolism; *retention* equals intake minus outgo (urine plus feces); absorption equals intake minus fecal outgo or retention plus urinary outgo.

In the studies of nutrition and chemical growth in childhood, daily nitrogen intakes which ranged from 8 to 13 gm. for children 4 to 12 years old, produced corresponding nitrogen absorption values ranging from 7 to 12 gm. (Table 56). The scatter diagrams in Figure 49 illustrate the difference between correlation of nitrogen intake with retention (0.47 ± 0.03) and with absorption (0.99 ± 0.01). The rather low correlation between intake and retention may be attributable to the fact that in well-nourished individuals, under conditions of more than adequate protein intake, the level of protein intake may not be the controlling factor in nitrogen retention. Under such circumstances the individual's growth capacity may be the dominating factor in controlling nitrogen retention. This is in accord with other evidence of the adequacy of the dietary regime, with respect to protein intake.

Older and larger children were given larger food intakes and as size and intake increased so did the quantities of nitrogen metabolized and excreted in urine. With an average daily intake of 473.0 ± 59.0 mg. of nitrogen per kilogram of body weight, nitrogen excretion in urine averaged 395.8 ± 55.1 mg. per day, 50.0 ± 10.5 mg. were found in the feces, 27.2 ± 22.5 mg. were retained and 423 mg. were absorbed. Five to eight per cent of the nitrogen intake was retained but 89 to 91 per cent was absorbed, demonstrating the care with which metabolic balances should be interpreted.

FAT

In order to measure fat absorption it is necessary to determine fecal lipids. Up to the present time there are two opposing schools of thought as to how this can best be done. Holt and coworkers[144] have assumed that, barring conditions of low fat intake, the most significant measure of fat absorption is the percentage of fat intake retained. Hutchison[162] is of the opinion that the percentage of fat in the dried stool is a more accurate measure of fat absorption, since he observed that the percentage of fat in dried feces was singularly constant, approximating one-third of the fecal dry weight. He concluded that this percentage of fat was physiologic for normal human feces, although the feces of dogs and rats were found to contain quite different percentages of fat.

TABLE 57

AVERAGES OF 502 AVERAGE DAILY FAT BALANCES FOR 18 CHILDREN*

Values in grams

Age group	Intake		Feces		Retention†	
year	mean	SD	mean	SD	mean	SD
4	71.18	8.32	1.59	0.39	69.59	8.07
5	67.36	7.20	2.09	0.51	65.27	6.99
6	74.14	11.03	1.98	0.63	72.16	10.79
8	91.29	13.72	2.71	0.85	88.58	13.11
9	82.76	2.26	3.04	0.87	79.72	2.73
10	112.81	2.25	3.72	0.55	109.09	2.84
11	112.81	2.25	3.05	0.35	109.76	2.50
12	112.82	2.25	3.82	0.26	109.00	2.36

* See Table 24, page 92 for body weights and lengths of subjects, distribution among age groups and number of balance periods for each group.

† Difference between fat intake and fat excretion in feces has been arbitrarily designated as fat retention.

One of the obvious advantages of using the percentage of fat in the stool, rather than the fat retention, to measure fat utilization, is the conservation of time and effort. It is a simple matter to determine the fat content of the stool, but to determine fat retention, it is necessary to conduct a balance experiment and accurately measure the fat contents of both the food and feces; generally, it is also necessary to restrict the activities of the subject.

Tables 57 and 58 record the fat balances determined for 18 normal children, ranging in age from 4 to 12 years. The mean daily intake of fat was 79.31 ± 16.58 gm. and the mean retention 77.01 ± 16.00 gm. An average of 97 per cent of the fat intake was retained and three per cent lost through the bowel. There was an average of 3.47 ± .44 gm. of fat consumed daily per kilogram of body weight of which 0.10 ± 0.03 gm. was excreted and 3.37 ± .44 gm. were retained.

TABLE 58

AVERAGES OF 502 AVERAGE DAILY FAT BALANCES FOR 18 CHILDREN

Values in grams per kilogram of body weight

Age group	Intake		Feces			Retention*		
year	mean	SD	mean	SD	per cent intake	mean	SD	per cent intake
4	3.95	.39	.09	.02	2	3.86	.31	98
5	3.60	.32	.11	.03	3	3.49	.30	97
6	3.39	.34	.09	.03	3	3.30	.42	97
8	3.47	.53	.10	.04	3	3.37	.55	97
9	2.90	.06	.11	.02	4	2.79	.15	96
10	3.45	.14	.11	.03	3	3.34	.12	97
11	3.15	.09	.08	.03	3	3.07	.11	97
12	2.69	.07	.09	.02	3	2.60	.18	97

* Difference between fat intake and fat excretion in feces has been arbitrarily designated as fat retention.

CALCIUM

Of all the chemical elements, calcium is most likely to be insufficient in the diets of human beings. The assimilation of this substance is a most popular subject of studies of growth and nutrition and is discussed in many, recent, comprehensive reviews.[312,330] The amount of calcium retained in any length of time depends upon factors involving the physiological status of the individual organism and upon chemical factors associated with the foods. About 98 per cent of the calcium used by growing children is deposited in the bones, consequently, calcium metabolism is chiefly concerned with the growth and maintenance of bone structure. An individual experiencing rapid skeletal growth or with a subnormal calcium concentration in bony tissues may require large amounts of this element to develop a satisfactory physiological state, although some studies indicate adjustment to reduced calcium intakes may occur.[251] Physiological and chemical factors may influence calcium utilization and the endocrine system superimposes an additional influence upon the amount of calcium that is used at any one time.

The average daily calcium balances for 29 children who were under observation for 2965 experimental days are given in Tables 59–63. The average daily intake per child during the 593 five-day

TABLE 59

AVERAGES OF 593 AVERAGE DAILY CALCIUM BALANCES FOR 29 CHILDREN*

Values in milligrams

Age group	Intake		Outgo				Retention‡		
			Urine		Feces				
year	mean	SD†	mean	SD†	mean	SD†	mean	SD†	per cent intake
4	829	106	109	37	503	80	217	154	26
5	867	293	88	26	607	171	172	147	20
6	823	233	73	24	596	132	154	136	19
8	1130	269	122	37	839	231	169	149	15
9	942	5	137	22	646	65	159	82	17
10	1197	491	105	38	700	228	392	262	33
11	947	25	74	17	711	39	162	56	17
12	947	25	159	14	618	58	170	60	18

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* In Table 20, page 87, are shown the distribution of the subjects among the age groups; the distribution of the five-day balance periods among the subjects; and, the average body weights and recumbent lengths of the subjects in each age group during the balance periods of study.

† The standard deviation (SD) was obtained with the formula: $\sqrt{\frac{\Sigma x^2 - N\bar{x}^2}{N-1}}$ in which N is the number of values, Σx^2 is the sum of the squares of the values, and $\bar{x}^2$ is the square of the mean of all the values.

‡ Retention is calculated as "Intake minus Outgo."

metabolic balance periods was 0.92 gm. (46 meq.). Of this amount approximately 0.74 gm. (37 meq.) were excreted in the urine and feces, producing an average daily retention of 0.18 gm. (9 meq.).

TABLE 60

DISTRIBUTION OF CALCIUM EXCRETION BETWEEN URINE AND FECES IN 593 CALCIUM BALANCES FOR 29 CHILDREN

Age group	EXCRETION OF CALCIUM INTAKE		TOTAL DAILY EXCRETION IN URINE AND FECES		
	Urine	Feces	Mean	Urine	Feces
year	per cent	per cent	mg.	per cent	per cent
4	13	61	612	18	82
5	10	70	695	13	87
6	9	72	669	11	89
8	11	74	961	13	87
9	14	69	783	17	83
10	9	58	805	13	87
11	8	75	785	9	91
12	17	65	777	20	80

Of the mean daily outgo, an average of 13 per cent was eliminated through the kidneys and 87 per cent by way of the bowel (Table 60). Of the calcium intake, the mean excretion in urine was 10 per cent and the mean fecal excretion was 70 per cent. The average retention of 20 per cent of their calcium intake, by these children, corresponds with results obtained by Outhouse, Mitchell and coworkers[37, 179, 331] on adults and children.

A temporary increase in storage may follow an increased in-

TABLE 61

AVERAGES OF 593 AVERAGE DAILY CALCIUM BALANCES FOR 29 CHILDREN

Values in milligrams per kilogram of body weight

Age group	Intake		Outgo				Retention	
			Urine		Feces			
year	mean	SD	mean	SD	mean	SD	mean	SD
4	44.9	3.5	6.0	2.0	27.5	5.4	11.4	7.4
5	46.0	14.4	4.7	1.4	32.4	9.1	8.9	7.6
6	37.6	10.0	3.4	1.0	27.3	6.1	6.9	6.0
8	43.3	10.6	4.6	1.3	32.1	9.2	6.6	5.8
9	33.0	1.3	4.8	0.7	22.6	2.2	5.6	2.8
10	35.7	13.7	3.2	1.0	20.9	6.5	11.6	7.5
11	26.5	0.7	2.1	0.4	19.9	0.7	4.5	1.6
12	22.6	1.1	3.8	0.4	14.8	0.8	4.0	1.6

take, whereas a change in dietary calcium to a level lower than before may in itself induce a negative balance, hence, observations of too-limited duration may result in erroneous conclusions concerning the adequacy of the dietary or the effect of growth, if interpretation is made without some knowledge of the nutrition and previous dietary of the subject. Neither the most satisfactory level of calcium intake, nor the optimal retention of calcium at any stage of infancy or childhood are known. From the results obtained on three groups of rats maintained on three different amounts of calcium in the food, Sherman and his students[14,306,351] have shown that the calcium content of the animal bodies at various ages was measureably influenced by the level

TABLE 62

AVERAGES OF 590 AVERAGE DAILY CALCIUM BALANCES FOR 27 CHILDREN

Values in milligrams per centimeter of recumbent length

Age group	Intake		Outgo				Retention	
			Urine		Feces			
year	mean	SD	mean	SD	mean	SD	mean	SD
4	7.5	0.9	1.0	0.3	4.6	0.7	1.9	1.3
5	7.8	2.6	0.8	0.2	5.5	1.6	1.5	1.3
6	6.9	2.0	0.6	0.2	5.0	1.2	1.3	1.2
8	8.7	2.1	0.9	0.2	6.5	1.9	1.3	1.2
9	6.9	0.3	1.0	0.2	4.7	0.4	1.2	0.6
10	8.6	3.8	0.8	0.3	5.0	1.8	2.8	2.0
11	6.8	0.2	0.5	0.2	5.1	0.4	1.2	0.4
12	6.0	0.2	1.0	0.1	3.9	0.4	1.1	0.4

TABLE 63

AVERAGES OF 593 AVERAGE DAILY CALCIUM BALANCES FOR 27 CHILDREN

Values in millieqnivalents

Age group	Intake	Outgo		Retention
		Urine	Feces	
year	mean	mean	mean	mean
4	41.3	5.4	25.0	10.9
5	43.3	4.4	30.3	8.6
6	41.1	3.6	29.8	7.7
8	56.4	6.1	41.8	8.5
9	47.0	6.8	32.3	7.9
10	59.7	5.4	34.8	19.5
11	47.3	3.7	35.5	8.1
12	47.3	7.9	30.9	8.5

of calcium intake. To what extent such differences exist among children remains to be determined.

In experimental work with animals the calcium to phosphorus ratio in the food intake has been demonstrated to have a significant bearing upon calcium metabolism.[215] Insufficient information on human subjects is available concerning the significance of Ca:P ratios of the food, in relation to the retention of calcium during growth. Upon a weight basis, with a mean daily Ca:P ratio of 1:1.4 in the food intake, the mean ratio in the urine was 1:7.1; in the feces, 1.6:1 and in the retention 1.0:1. The wide variations in the Ca:P ratios are shown by the ranges of ratios, given in Table 64.

TABLE 64

CALCIUM TO PHOSPHORUS RATIOS OF AVERAGES OF 593 AVERAGE DAILY BALANCES FOR 29 CHILDREN

Age	RATIOS BASED ON GRAMS PER DAY				RATIOS BASED ON MILLIEQUIVALENTS PER DAY			
	Intake	Urine	Feces	Retention	Intake	Urine	Feces	Retention
4	.73	.17	1.62	1.15	.62	.14	1.39	1.00
5	.75	.14	1.71	1.06	.65	.12	1.47	0.91
6	.73	.12	1.50	1.09	.63	.10	1.29	0.93
8	.80	.15	1.95	0.88	.68	.13	1.67	0.76
9	.64	.15	1.53	1.10	.55	.13	1.31	0.94
10	.70	.13	1.33	1.09	.60	.11	1.14	0.94
11	.63	.08	1.54	1.33	.54	.07	1.32	1.16
12	.63	.16	1.56	1.30	.54	.14	1.34	1.13

Whatever the cause of the variations in response of the children in these studies, all of whom were considered to be in excellent nutritive state and health and were given some degree of adjustment preceding each series of observations, there were variations in the individuals' calcium storage which cannot be accounted for by level of calcium intake. The differences in ability to retain calcium may be explained by the need for a longer period of preparation of the subjects, for it has been shown that children become more uniform in their ability to store minerals after six or eight months on a metabolic regime (p. 40).

When the 29 children are compared on the basis of average calcium intake per day (Table 65) and per kilogram of body weight per day (Table 66) there were greater retentions with the higher intakes of calcium although the percentages of intake retained do not evidence a consistent relationship. Because calcium metabolism is largely concerned with skeletal growth and development in childhood the assumption is frequently made that calcium retention is related to stature. Table 62 records the average daily calcium balances calculated on the basis of centimeters of recumbent length. With an average daily calcium intake of 7.7 ± 2.3 mg. per centimeter there was an average daily elimination by way of the kidney and bowel of 0.8 ± 0.3 and 5.4 ± 1.6 mg. per centimeter, respectively, resulting in an average retention of 1.5 ± 1.3 mg. per centimeter. As a comparison of the calcium intakes and retentions per kilogram of body weight and per centimeter of recumbent length, the average daily figures for each balance period are shown in the scatter diagrams in Figure 50.

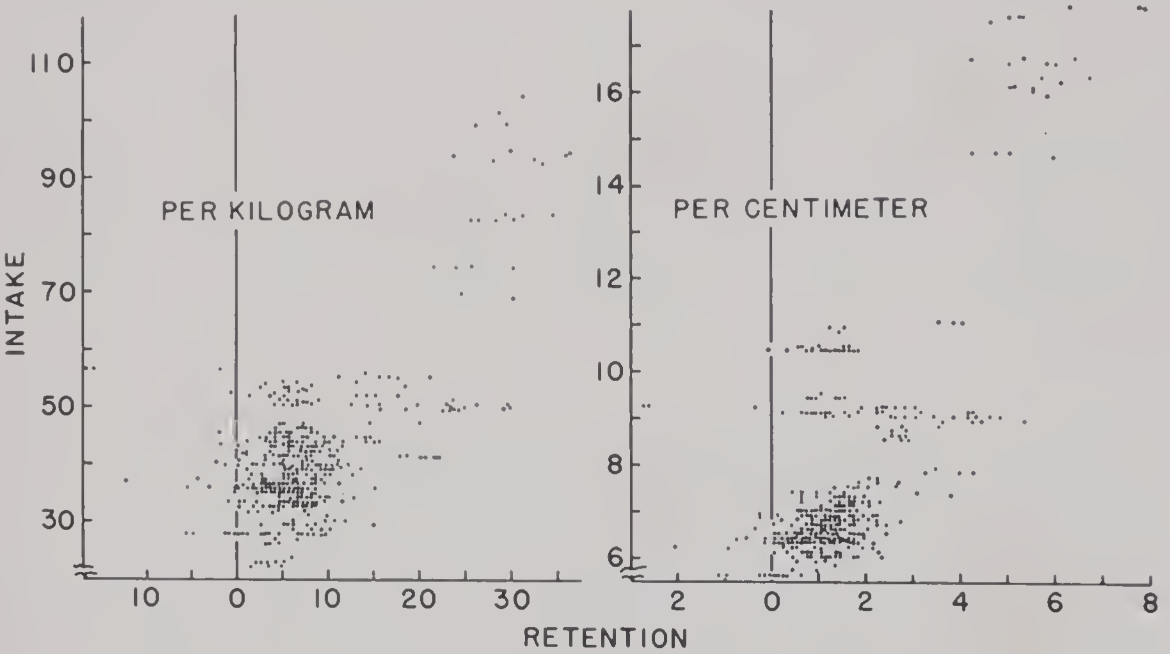

Figure 50. Calcium intakes *vs.* retentions; in milligrams per kilogram of body weight and per centimeter of recumbent length.

There is some evidence in the literature which indicates that the vegetable fiber content of the diet may interfere with the absorption of calcium. However, a study of the correlation coefficients between fecal calcium and total complex carbohydrate in the feces of the children indicates an extremely slight correlation (0.06 ± 0.05 as determined from 414 five-day balances). The influence of fiber containing foods on calcium retention seems to indicate little, if any, connection between calcium excretion and fiber *per se*. The type of mixture or the distribution of cellulose, hemicellulose and lignin in the food may have greater significance than the total fiber content alone.[305] But greater variations in fiber and calcium levels might reveal this, in other experiments.

The existence of soap in the feces has long aroused interest since it was surmised that the amount of soap excreted might be related to calcium intake. We have observed the same children on 400 gm. of milk daily, followed by periods at an 800 gm. level, each superimposed upon a generous diet. The fat content of the stools were increased from 12.0 to 16.0 per cent of the dry stool by the increased milk intake. Less than 2 gm. of fecal fat were eliminated with the lower intake of milk and over 3 gm. with the larger intake. There was likewise a marked increase in soaps in the feces during the larger milk intake. The greater part of the increased fecal fat excretion seemed to be due to the excess soap and this in turn to the augmented calcium intake. Fecal calcium increased from 0.62 grams to 1.01 gm. with the enriched diet.

TABLE 65

RELATION BETWEEN CALCIUM INGESTION AND CALCIUM RETENTION BASED UPON AVERAGE DAILY INTAKE AND RETENTION

(593 balances on 29 children)

Calcium ingested per day	Children	Five-day balances determined	MEAN			Mean surface area	CALCIUM RETAINED		
			Age	Height	Weight		Mean	SD	Of intake
mg.	number	number	months	cm.	kg.	sq.m.	mg./day		per cent
1700–1800	1	10	123	134.4	34.30	1.116	696	272	40
1500–1600	1	14	79	116.6	22.73	0.837	517	192	34
1400–1500	1	13	68	113.2	20.12	0.778	489	185	33
1300–1400	1	11	106	128.9	25.46	0.957	449	194	32
1100–1200	3	100	91	125.9	24.92	0.916	144	131	12
900–1000	13	100	111	134.3	29.78	1.065	247	136	26
700– 800	9	345	71	115.1	20.19	0.773	125	61	16

TABLE 66

AVERAGE DAILY CALCIUM INGESTION, RETENTION AND ABSORPTION PER KILOGRAM OF BODY WEIGHT

Calcium ingested	Children	Five-day balances	CALCIUM RETAINED		
			Mean	SD	Of intake
mg./kg.	number	number	mg./kg.		per cent
70–80	1	13	24.2	8.9	33
60–70	2	33	17.9	11.0	28
50–60	4	25	18.2	7.3	34
40–50	9	224	7.1	5.7	16
30–40	9	254	5.9	3.0	16
20–30	4	44	6.0	2.6	22

There are divergencies in the amount of calcium retained from any quantity ingested, under similar experimental procedures. This indicates that there are potent factors other than level of intake affecting the utilization of calcium (Figure 51).

The levels of calcium and phosphorus intake are of major importance in the retentions of either of these elements. Of equal significance is the presence of adequate amounts of vitamin D[166] and ascorbic acid, for these are essential in effecting the most efficient utilization of calcium and phosphorus and are of major importance in maintaining healthy bone structure. Although the quantities of these vitamins necessary in childhood can only be approximated at the present time the diets of the children contained an average of 112 mg. of ascorbic acid, a more generous quota than the 50 to 75 mg. recently recommended by the Committee on Food and Nutrition, National Research Council. The diets of the children included liberal quantities of milk, eggs, butter, fresh green vegetables and irradiated milk. Not only was the food a good source of vitamin D but the children lived in the country and were amply exposed to sunshine. The lower mean daily calcium retention of 7.9 ± 6.8 mg. per kilogram of body weight by these children is further evidence of their well-filled calcium stores, as well as the high nutritive value of the diets, although the retention is lower than the 12 mg. which Shohl and others have estimated to be necessary in childhood.

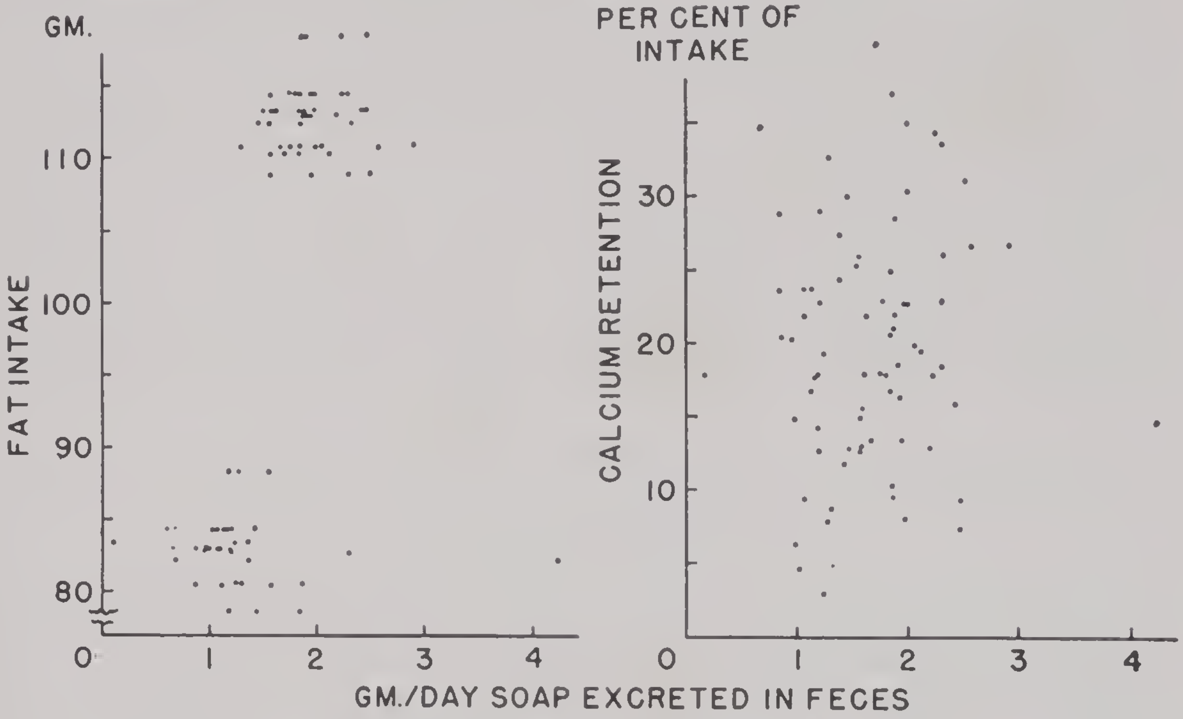

Figure 51. Soap excreted in feces *vs.* fat intake and calcium retention.

PHOSPHORUS

Phosphorus is widely distributed in the body, combined in soft and hard tissues and associated with protein, carbohydrate, fats, and other substances involved in metabolism. Approximately 30 per cent of the retained phosphorus is combined in nitrogen containing substances, while calcium combines with the remaining 70 per cent. Although phosphorus is essential in the metabolism of fats and carbohydrates, and necessary for activity, only a small portion of the intake is needed in these processes. Because phosphorus participates in so many diverse phases of metabolism and the electroneutrality of the body, it is difficult to interpret phosphorus balances satisfactorily.

The average daily phosphorus balances are shown in Tables 67–70. The mean daily intake of phosphorus for childhood amounted to 1250 ± 294 mg. (72.5 meq.), of which 684 ± 152 mg. (39.7 meq.) and 393 ± 112 mg. (22.8 meq.) were excreted by the kidneys and bowels, respectively, and 173 ± 175 mg. (10.0 meq.) were retained. On the average, only 14 per cent of the consumed phosphorus was retained, 55 per cent was excreted in the urine and 31 per cent lost in the feces.

TABLE 67

AVERAGES OF 593 AVERAGE DAILY PHOSPHORUS BALANCES FOR 29 CHILDREN*

Values in milligrams

Age group	Intake		Outgo				Retention‡		
			Urine		Feces				
year	mean	SD†	mean	SD†	mean	SD†	mean	SD†	per cent intake
4	1141	137	642	82	311	54	188	175	17
5	1152	281	635	96	355	112	162	167	14
6	1130	220	591	93	398	113	141	126	13
8	1416	225	794	161	431	95	191	180	14
9	1464	34	897	62	423	73	144	78	10
10	1714	444	829	103	527	90	358	343	21
11	1501	35	918	34	461	32	122	75	8
12	1501	35	975	21	395	38	131	64	9

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[*] In Table 20, page 87, are shown the distribution of the subjects among the age groups; the distribution of the five-day balance periods among the subjects; and, the average body weights and recumbent lengths of the subjects in each age group during the balance periods of study.

[†] The standard deviation (SD) was obtained with the formula: $\sqrt{\frac{\Sigma x^2 - N\bar{x}^2}{N-1}}$ in which N is the number of values, Σx^2 is the sum of the squares of the values, and $\bar{x}^2$ is the square of the mean of all the values.

[‡] Retention is calculated as "Intake minus Outgo."

Elimination of the larger portion of the outgo of phosphorus by the kidneys, is in accord with the fact that the element is used widely in metabolism. In contrast to the 70 per cent average loss of calcium intake through the alimentary canal, only 26 to 35 per cent of the total intake of phosphorus occurs by that route. The remaining 48 to 65 per cent of the phosphorus lost from the body occurs as a metabolite in the urine. The average daily phosphorus intake per kilogram of body weight for childhood amounted to 55 mg.; the average daily retention was approximately 8 mg. per kilogram of body weight per day.

TABLE 68

DISTRIBUTION OF PHOSPHORUS EXCRETION BETWEEN URINE AND FECES IN 593 PHOSPHORUS BALANCES FOR 29 CHILDREN

Age group	EXCRETION OF PHOSPHORUS INTAKE		TOTAL DAILY EXCRETION IN URINE AND FECES		
	Urine	Feces	Mean	Urine	Feces
year	per cent	per cent	mg.	per cent	per cent
4	56	27	953	67	33
5	55	31	990	64	36
6	52	35	989	60	40
8	56	30	1225	65	35
9	61	29	1320	68	32
10	48	31	1356	61	39
11	61	31	1379	67	33
12	65	26	1370	71	29

TABLE 69

AVERAGES OF 593 AVERAGE DAILY PHOSPHORUS BALANCES FOR 29 CHILDREN

Values in milligrams per kilogram of body weight

Age group	Intake		Outgo				Retention	
			Urine		Feces			
year	mean	SD	mean	SD	mean	SD	mean	SD
4	61.7	5.1	34.9	5.3	16.9	3.0	9.9	8.7
5	61.3	13.4	33.8	4.8	18.9	5.7	8.6	8.6
6	51.7	8.7	27.1	3.9	18.2	5.2	6.4	5.6
8	54.3	9.0	30.3	5.6	16.6	3.8	7.4	7.2
9	51.2	3.1	31.4	1.7	14.8	2.6	5.0	2.9
10	51.3	11.9	24.9	3.4	15.8	2.7	10.6	9.8
11	42.0	1.0	25.7	0.1	12.9	0.9	3.4	2.1
12	35.8	2.2	23.3	0.6	9.4	1.2	3.1	1.6

TABLE 70

AVERAGES OF 593 AVERAGE DAILY PHOSPHORUS BALANCES FOR 29 CHILDREN*

Values in milliequivalents

Age group	Intake	Outgo		Retention
		Urine	Feces	
year	mean	mean	mean	mean
4	66.3	37.4	18.0	10.9
5	66.9	36.9	20.6	9.4
6	65.7	34.3	23.2	8.2
8	82.3	46.1	25.1	11.1
9	85.1	52.1	24.6	8.4
10	99.6	48.2	30.6	20.8
11	87.3	53.4	26.9	7.0
12	87.3	56.7	23.1	7.5

* Valence of 1.8 has been used in calculating phosphorus in milliequivalents.

MAGNESIUM

Magnesium is associated with calcium in skeletal formation and is present in the soft tissues and body fluids, yet knowledge of its specific physiological function in human nutrition is meager. Most mineral balance studies include determination of magnesium because it is associated with calcium, but the results usually appear erratic and uninterpretable. Some of the results are due to unsatisfactory chemical procedures for accurately determing magnesium in biological materials. Numerous elements, especially iron, may interfere with the quantitative determination of magnesium and often these contribute to the confused interpretations.

The average daily magnesium balances determined for 29 children during 593 five-day experimental periods are given in Tables 71–74. The mean daily magnesium intake for the group was 297 ± 42 mg. (24.4 meq.). Of this daily intake 88 ± 18 mg. (7.2 meq.) and 162 ± 39 mg. (13.3 meq.) were excreted in the urine and feces, respectively, the mean retention was 47 ± 47 gm. (3.9 meq.).

TABLE 71

AVERAGES OF 593 AVERAGE DAILY MAGNESIUM BALANCES FOR 29 CHILDREN*

Values in milligrams

Age group	Intake		Outgo				Retention‡		
			Urine		Feces				
year	mean	SD†	mean	SD†	mean	SD†	mean	SD†	per cent intake
4	290	18	74	15	168	22	48	28	16
5	284	43	83	15	146	46	55	65	19
6	287	38	91	21	162	34	34	30	12
8	316	38	84	18	174	33	58	44	18
9	291	6	108	22	158	21	25	20	9
10	356	92	110	22	182	34	64	65	18
11	304	10	93	20	185	15	26	27	9
12	304	10	93	18	178	18	33	28	11

* In Table 20, page 87, are shown the distribution of the subjects among the age groups; the distribution of the five-day balance periods among the subjects; and, the average body weights and recumbent lengths of the subjects in each age group during the balance periods of study.

† The standard deviation (SD) was obtained with the formula: $\sqrt{\frac{\Sigma x^2 - N\bar{x}^2}{N-1}}$ in which N is the number of values, Σx^2 is the sum of the squares of the values, and $\bar{x}^2$ is the square of the mean of all the values.

‡ Retention is calculated as "Intake minus Outgo."

Of the mean magnesium excretion of the 29 children, 250 mg. per day, 35 per cent appeared in the urine and the remaining 65 per cent was eliminated through the bowel. An average of only 16 per cent of the magnesium intake was retained by the body while 30 per cent of it was excreted by the kidneys and 54 per cent by the gastrointestinal tract. Apparently, these children had a very low magnesium requirement. Assuming that magnesium metabolism may be related to body mass, these children, with an average daily allowance of 13 mg. of magnesium per kilogram of body weight, retained only 2 mg. per kilogram of body weight for growth. High urinary excretion indicates that the subjects had a large additional amount of magnesium (twice

TABLE 72

DISTRIBUTION OF MAGNESIUM EXCRETION BETWEEN URINE AND FECES IN 593 MAGNESIUM BALANCES FOR 29 CHILDREN

Age group	EXCRETION OF MAGNESIUM INTAKE		TOTAL DAILY EXCRETION IN URINE AND FECES		
	Urine	Feces	Mean	Urine	Feces
year	per cent	per cent	mg.	per cent	per cent
4	26	58	242	31	69
5	29	52	229	36	64
6	32	56	253	36	64
8	27	55	258	33	67
9	37	54	266	41	59
10	31	51	292	38	62
11	30	61	278	33	67
12	31	58	271	34	66

TABLE 73

AVERAGES OF 593 AVERAGE DAILY MAGNESIUM BALANCES FOR 29 CHILDREN

Values in milligrams per kilogram of body weight

Age group	Intake		Outgo				Retention	
			Urine		Feces			
year	mean	SD	mean	SD	mean	SD	mean	SD
4	15.7	1.0	4.0	0.9	9.1	1.6	2.6	1.4
5	15.1	1.4	4.4	0.9	7.9	2.6	2.8	3.3
6	13.1	1.7	4.2	0.8	7.4	1.6	1.5	1.4
8	12.1	1.6	3.2	0.8	6.7	1.4	2.2	1.6
9	10.2	0.6	3.8	0.6	5.5	1.0	0.9	0.7
10	10.6	2.7	3.3	0.6	5.4	1.2	1.9	1.9
11	8.5	0.3	2.6	0.4	5.2	0.3	0.7	0.8
12	7.2	1.0	2.2	0.6	4.2	0.7	0.8	0.7

as large as the retention figure) available for use in physiological processes in which magnesium participates. Magnesium recently has been shown to function as an essential component of the enzyme system involved in the breakdown of carbohydrate in muscle metabolism.[53a]

TABLE 74

AVERAGES OF 593 AVERAGE DAILY MAGNESIUM BALANCES FOR 29 CHILDREN

Values in milliequivalents

Age group	Intake	Outgo		Retention
		Urine	Feces	
year	mean	mean	mean	mean
4	23.8	6.1	13.8	3.9
5	23.3	6.8	12.0	4.5
6	23.6	7.5	13.3	2.8
8	26.0	6.9	14.3	4.8
9	23.9	8.9	13.0	2.0
10	29.3	9.0	15.0	5.3
11	25.0	7.7	15.2	2.1
12	25.0	7.7	14.6	2.7

SULFUR

In studies of the metabolism of sulfur more emphasis has been placed upon the sulfur partition of the urine than upon the balance between intake and outgo. This is especially true with respect to investigations of children. The studies of the Laboratory have included observations of both types, but only the balance data are presented in Tables 75–78.

TABLE 75
AVERAGES OF 593 AVERAGE DAILY SULFUR BALANCES FOR 29 CHILDREN*

Values in milligrams

Age group	Intake		Outgo				Retention‡			Absorption§
			Urine		Feces					
year	mean	SD†	mean	SD†	mean	SD†	mean	SD†	per cent intake	mean
4	684	49	520	98	78	21	86	118	13	606
5	679	78	525	52	86	20	68	76	10	593
6	692	63	575	42	77	19	40	68	6	615
8	808	85	657	108	92	22	59	92	7	716
9	973	61	732	25	135	17	106	63	11	838
10	1027	126	731	92	120	22	176	122	17	907
11	999	55	772	21	133	11	94	69	10	866
12	1000	55	760	22	137	10	103	66	10	863

* In Table 20, page 87, are shown the distribution of the subjects among the age groups; the distribution of the five-day balance periods among the subjects; and, the average body weights and recumbent lengths of the subjects in each age group during the balance periods of study.

† The standard deviation (SD) was obtained with the formula: $\sqrt{\frac{\Sigma x^2 - N\bar{x}^2}{N-1}}$ in which N is the number of values, Σx^2 is the sum of the squares of the values, and $\bar{x}^2$ is the square of the mean of all the values.

‡ Retention is calculated as "Intake minus Outgo."

§ Absorption is calculated as "Intake minus Feces."

The mean sulfur intake during childhood amounted to 750 ± 130 mg. per day. Of the mean daily intake an average daily loss of 593 ± 104 mg. occurred through the urine (79 per cent of the intake) and 89 ± 26 mg. (12 per cent of the intake) through the stools. A mean of only 68 ± 90 mg. of sulfur (nine per cent of the intake) was retained per day. Of the mean daily sulfur intake of 750 mg., 661 mg. (88 per cent of the intake) was absorbed. From Freyberg and Grant's work on adults[107] it is possible that a portion of the retained sulfur was lost through the skin, although in childhood the sulfur demand for growth is high and may be responsible for a different distribution of the sulfur excretion.

Sulfur and nitrogen have similar metabolic patterns. Although

87 per cent of the total outgo of sulfur leaves the body by way of the kidney, some of the 13 per cent excreted in the stools may be of metabolic origin.

TABLE 76

DISTRIBUTION OF SULFUR EXCRETION BETWEEN URINE AND FECES IN 593 SULFUR BALANCES FOR 29 CHILDREN

Age group	EXCRETION OF SULFUR INTAKE		TOTAL DAILY EXCRETION IN URINE AND FECES		
	Urine	Feces	Mean	Urine	Feces
year	per cent	per cent	mg.	per cent	per cent
4	76	11	598	87	13
5	77	13	611	86	14
6	83	11	652	88	12
8	81	12	749	88	12
9	75	14	867	84	16
10	71	12	851	86	14
11	77	13	905	85	15
12	76	14	897	85	15

A large proportion of the sulfur retained in the body is laid down in the soft tissues and the organic matrix of the bone. The mean daily intake per kilogram of body weight during childhood was 33.1 ± 4.1 mg. of which 2.9 ± 3.9 mg. and 29.2 mg., respectively, were retained and absorbed. The younger children received more sulfur in their diets, on a basis of per kilogram of body weight, than did the older ones. The higher levels of intake were parallelled by increased absorption.

TABLE 77

AVERAGES OF 593 AVERAGE DAILY SULFUR BALANCES FOR 29 CHILDREN

Values in milligrams per kilogram of body weight

Age group	Intake		Outgo				Retention		Absorption
			Urine		Feces				
year	mean	SD	mean	SD	mean	SD	mean	SD	mean
4	37.1	1.6	28.5	6.1	4.2	1.1	4.4	6.1	32.9
5	36.2	3.4	28.0	3.2	4.6	0.9	3.6	4.0	31.6
6	31.6	1.8	26.4	2.8	3.5	0.8	1.7	3.1	28.1
8	30.9	3.0	25.1	4.1	3.5	1.0	2.3	3.7	27.4
9	34.0	1.5	25.6	0.8	4.7	0.7	3.7	2.3	29.3
10	30.8	3.3	22.0	3.4	3.6	0.6	5.2	3.5	27.2
11	27.9	0.3	21.6	0.7	3.7	0.5	2.6	2.0	24.2
12	23.9	0.8	18.2	0.7	3.3	0.3	2.4	1.7	20.6

Only two naturally occurring amino acids containing sulfur are known. Experiments with these two amino acids, cystine and methionine, indicate that in rats methionine is an essential amino acid but cystine is not. However, cystine is non-essential only when an adequate supply of methionine is furnished to cover the whole sulfur need. Cystine fed in the presence of restricted methionine intake can go far in assisting the limited methionine in satisfying body requirements. As yet we do not know with certainty that these facts established on lower mammals can be applied to human nutrition.[282,284]

TABLE 78

AVERAGES OF 593 AVERAGE DAILY SULFUR BALANCES FOR 29 CHILDREN*

Values in milliequivalents

Age group	Intake	Outgo		Retention	Absorption
		Urine	Feces		
year	mean	mean	mean	mean	mean
4	42.7	32.4	4.9	5.4	37.8
5	42.4	32.8	5.4	4.2	37.0
6	43.2	35.9	4.8	2.5	38.4
8	50.4	41.0	5.7	3.7	44.7
9	60.7	45.7	8.4	6.6	52.3
10	64.1	45.6	7.5	11.0	56.6
11	62.3	48.1	8.3	5.9	54.0
12	62.3	47.4	8.5	6.4	53.8

* Valence of 2 has been used in calculating sulfur in milliequivalents.

POTASSIUM, SODIUM, AND CHLORINE

Sodium, potassium and chlorine are often considered together, because they are involved in the distribution of body water into fractions composing the intracellular and extracellular fluids. Excessive proportions of potassium in diets prevent the full utilization of sodium by the organism, and may cause insufficient assimilation and retention of other elements, especially calcium and phosphorus. For these reasons, the ratio of potassium to sodium has received more attention in nutrition and growth than have the absolute allowances of each. As a matter of fact, in planning average diets the amounts of these elements are seldom considered, because it is generally assumed that a diet which is adequate in all other respects will contain sufficient amounts of sodium and potassium to meet the nutritive requirements of the body. In certain types of metabolic disturbances it is not only important that the total daily allowance of these two elements be considered, but also the relation of them to chlorine. The body adjusts itself slowly to a change in level of intake of sodium, potassium, and chlorine. Instead of the generally accepted metabolic adjustment of a few days, it may take two weeks to a month, or longer, for equilibrium to become established, even with dietaries and environmental conditions constant.

Potassium, sodium and chlorine are excreted in significant amounts through the skin. The measurement of skin excretion is time-consuming and difficult and cannot be determined at the present time without abnormal restriction of activity and environment of the experimental subject. The results presented for sodium, potassium and chlorine contents of food, urine and feces are accurate analytical values, but the retention values are only rough approximations. It has been shown in the adult that approximately 14 per cent of the retained sodium is lost through the skin.[107] In infants[336] it has been shown that 30 per cent or more of potassium and about 15 per cent of chlorine may be excreted through the skin.

Potassium

Potassium is associated with nitrogen in the formation of soft tissue in the body; this by virtue of its activity in relation to cellular water. When nitrogen is stored in the form of new protoplasmic protein, potassium is retained in sufficient quantities to meet the intracellular fluid needs of the newly formed cells. Potassium is known to be held within the cell, although the factors controlling its entrance into and its egress from the cell are unknown.[312] The element participates in the control of body neutrality and in this capacity it occurs in conjunction with phosphate, chloride and bicarbonate. Potassium, like other elements, also acts to regulate and maintain neuromuscular irritability.

TABLE 79

AVERAGES OF 593 AVERAGE DAILY POTASSIUM BALANCES FOR 29 CHILDREN*

Values in milligrams

Age group	Intake		Outgo				Retention‡		
			Urine		Feces				
year	mean	SD†	mean	SD†	mean	SD†	mean	SD†	per cent intake
4	2565	211	2147	342	311	98	107	444	4
5	2574	323	1983	301	404	127	187	282	7
6	2629	315	2043	258	383	127	203	176	8
8	2955	363	2364	396	339	113	252	237	9
9	3252	46	2629	161	331	98	292	126	9
10	3560	505	2779	395	518	254	263	354	7
11	3487	47	2853	142	350	38	284	132	8
12	3487	47	2878	252	263	35	346	268	10

* In Table 20, page 87, are shown the distribution of the subjects among the age groups; the distribution of the five-day balance periods among the subjects; and, the average body weights and recumbent lengths of the subjects in each age group during the balance periods of study.

† The standard deviation (SD) was obtained with the formula: $\sqrt{\frac{\Sigma x^2 - N\bar{x}^2}{N-1}}$ in which N is the number of values, Σx^2 is the sum of the squares of the values, and $\bar{x}^2$ is the square of the mean of all the values.

‡ Retention is calculated as "Intake minus Outgo."

TABLE 80

DISTRIBUTION OF POTASSIUM EXCRETION BETWEEN URINE AND FECES IN 593 POTASSIUM BALANCES FOR 29 CHILDREN

Age group	EXCRETION OF POTASSIUM INTAKE		TOTAL DAILY EXCRETION IN URINE AND FECES		
	Urine	Feces	Mean	Urine	Feces
year	per cent	per cent	mg.	per cent	per cent
4	84	12	2458	87	13
5	77	16	2387	83	17
6	78	14	2426	84	16
8	80	11	2703	87	13
9	81	10	2960	89	11
10	78	15	3297	84	16
11	82	10	3203	89	11
12	82	8	3141	92	8

The mean daily potassium intake for 29 children (Tables 79–81) amounted to 2776 ± 437 mg. (71.0 meq.) of which 2191 ± 405 mg. (56.0 meq.) appeared in the urine and 376 ± 136 mg. (9.7 meq.) were excreted in the stools. The mean daily retention was 209 ± 268 mg. (or 5.3 meq.). The large standard deviations accompanying the potassium figures illustrate the wide differences among individuals which must be expected in balances of such approximate nature.

TABLE 81

AVERAGES OF 593 AVERAGE DAILY POTASSIUM BALANCES FOR 29 CHILDREN

Values in milliequivalents

Age group	Intake	Outgo		Retention
		Urine	Feces	
year	mean	mean	mean	mean
4	65.7	55.0	8.0	2.7
5	65.9	50.8	10.3	4.8
6	67.3	52.3	9.8	5.2
8	75.6	60.5	8.7	6.4
9	83.3	67.3	8.5	7.5
10	91.1	71.1	13.3	6.7
11	89.3	73.0	9.0	7.3
12	89.3	73.7	6.7	8.9

An average of 79 per cent of the intake of potassium was excreted by the kidneys, 13 per cent by the bowel and less than eight per cent was retained. Of that leaving the body through the intestinal tract, a large portion may be combined with fatty acids in the form of potassium soaps. It must be remembered that the retention value includes the potassium lost through the skin. If 30 per cent of the retention figure were lost through the skin then approximately 146 mg. of potassium were actually retained, on the average, per day during childhood. This quantity of potassium, particularly during rapid growth, is comparatively small.

Sodium

Sodium is found chiefly in the plasma and the extracellular fluids of the body although some is associated with cartilage and muscle cells. It functions largely with chloride and bicarbonate in control of the concentration of the body fluids. It is the most predominant positive mineral element in the extracellular fluids.[209] Sodium salts are readily absorbed and circulated throughout the entire body. The actual allowances of sodium and potassium per day are comparatively small but the ratio between these two elements in the diet is considered of greatest practical importance. The high proportion of potassium in comparison with sodium in most common foods, introduces two possible dangers which may be evidenced metabolically by preventing full utilization of the sodium by the body or by causing insufficient assimilation and retention of other elements. To compensate for the excess amounts of potassium over sodium which naturally occur in foods and to enhance palatability, sodium chloride is added to many foods incorporated in the daily diet.

TABLE 82

AVERAGES OF 593 AVERAGE DAILY SODIUM BALANCES FOR 29 CHILDREN*

Values in milligrams

Age group	Intake		Outgo				Retention‡		
			Urine		Feces				
year	mean	SD†	mean	SD†	mean	SD†	mean	SD†	per cent intake
4	2128	192	1729	406	41	32	358	412	17
5	2101	248	1828	242	54	48	219	260	10
6	2162	192	1981	205	34	28	147	207	7
8	2471	250	2138	343	46	46	287	242	12
9	2997	56	2691	78	26	17	280	101	9
10	3009	221	2465	464	32	26	512	411	17
11	3013	45	2738	83	58	22	217	86	7
12	3013	45	2524	121	19	7	470	143	15

* In Table 20, page 87, are shown the distribution of the subjects among the age groups; the distribution of the five-day balance periods among the subjects; and, the average body weights and recumbent lengths of the subjects in each age group during the balance periods of study.

† The standard deviation (SD) was obtained with the formula: $\sqrt{\frac{\Sigma x^2 - N\bar{x}^2}{N-1}}$ in which N is the number of values, Σx^2 is the sum of the squares of the values, and $\bar{x}^2$ is the square of the mean of all the values.

‡ Retention is calculated as "Intake minus Outgo."

The mean daily intake of sodium for 29 children amounted to 2310 ± 368 mg. (100.5 meq.), of which 787 mg. were given in chemically pure sodium chloride and the remainder in the foods (Tables 82–84). Sodium is not stored to any large extent in the body and, because of its distribution throughout the extracellular water, utilization and excretion take place relatively rapidly. The average daily excretion of sodium in the urine amounted to 2022 ± 377 mg. (88.0 meq.), while a small (42 ± 39 mg. or 1.8

meq.) but significant quantity was eliminated through the alimentary canal.

The mean sodium retention was 246 ± 280 mg. (10.7 meq.). Assuming that approximately 12 per cent of the retained sodium was dissipated through the skin, then the mean daily retention figure is about 30 mg. too high, giving a corrected value of 216 mg. per day. Coexistent with a mean daily retention of 216 mg. of this essential element, an average of 2268 mg. (98.7 meq.), were absorbed. Eighty-seven per cent of the sodium intake found its way into the urine, 2 per cent into the feces and 11 per cent

TABLE 83

DISTRIBUTION OF SODIUM EXCRETION BETWEEN URINE AND FECES IN 593 SODIUM BALANCES FOR 29 CHILDREN

Age group	EXCRETION OF SODIUM INTAKE		TOTAL DAILY EXCRETION IN URINE AND FECES		
	Urine	Feces	Mean	Urine	Feces
year	per cent	per cent	mg.	per cent	per cent
4	81	2	1770	98	2
5	87	3	1882	97	3
6	92	1	2015	98	2
8	86	2	2184	98	2
9	90	1	2717	99	1
10	82	1	2497	99	1
11	91	2	2796	98	2
12	84	1	2543	99	1

TABLE 84

AVERAGES OF 593 AVERAGE DAILY SODIUM BALANCES FOR 29 CHILDREN

Values in milliequivalents

Age group	Intake	Outgo		Retention
		Urine	Feces	
year	mean	mean	mean	mean
4	92.6	75.2	1.8	15.6
5	91.4	79.5	2.4	9.5
6	94.0	86.2	1.5	6.3
8	107.5	93.0	2.0	12.5
9	130.3	117.0	1.1	12.2
10	130.9	107.2	1.4	22.3
11	131.0	119.1	2.5	9.4
12	131.0	109.8	0.8	20.4

was retained. Of the total sodium excretion, 98 per cent was eliminated through the kidneys and 2 per cent by way of the feces. The large urinary excretion is to be expected since sodium serves generally in the metabolic processes of the body in connection with the maintenance of electro-neutrality of the body through the extracellular fluid of the body and the blood. The sodium to potassium ratios for the 29 children are given in Table 85.

TABLE 85

SODIUM TO POTASSIUM RATIOS OF THE AVERAGES OF 593 AVERAGE DAILY SODIUM AND POTASSIUM BALANCES FOR 29 CHILDREN

Age	BASED ON GRAMS PER DAY				BASED ON MILLIEQUIVALENTS PER DAY			
	Intake	Urine	Feces	Retention	Intake	Urine	Feces	Retention
4	.83	0.80	.13	3.34	1.41	1.37	0.22	5.78
5	.82	0.92	.13	1.17	1.39	1.56	0.23	1.98
6	.82	0.97	.09	0.72	1.40	1.65	0.15	1.21
8	.84	0.90	.14	1.14	1.42	1.54	0.23	1.95
9	.92	1.02	.08	0.96	1.56	1.74	0.13	1.63
10	.84	0.89	.06	1.95	1.44	1.51	0.10	3.33
11	.86	0.96	.16	0.76	1.47	1.63	0.28	1.29
12	.86	0.88	.07	1.36	1.47	1.49	0.12	2.29

Chlorine

Chlorine is found distributed throughout the body and is contained in all body secretions and excretions. It is stored, to a

TABLE 86

AVERAGES OF 593 AVERAGE DAILY CHLORINE BALANCES FOR 29 CHILDREN

Values in milliequivalents

Age group	Intake	Outgo		Retention
		Urine	Feces	
year	mean	mean	mean	mean
4	94.7	82.9	1.6	10.2
5	93.8	84.8	1.8	7.2
6	96.9	89.6	1.4	5.9
8	107.9	96.7	1.6	9.6
9	122.3	114.8	0.9	6.6
10	125.1	112.5	1.1	11.5
11	122.9	118.4	1.6	2.9
12	122.9	107.8	0.7	14.4

limited extent, in the skin, subcutaneous tissue and skeleton. The chlorides of the blood, particularly sodium chloride, comprise about two-thirds of the blood anions which serve an important role in the electroneutrality relationships within the body. Sodium and chlorine serve in large measure to maintain the osmotic pressure of the extracellular fluids. Gastric secretion contains chlorine as free hydrochloric acid and in the form of salts. Few elements serve a more varied or important physiological role than does chlorine.

TABLE 87

AVERAGES OF 593 AVERAGE DAILY CHLORINE BALANCES FOR 29 CHILDREN*

Values in milligrams

Age group	Intake		Outgo				Retention‡		
			Urine		Feces				
year	mean	SD†	mean	SD†	mean	SD†	mean	SD†	per cent intake
4	3360	234	2941	286	56	30	363	221	11
5	3324	344	3009	338	63	33	252	195	8
6	3437	282	3176	295	50	29	211	202	6
8	3826	316	3428	409	58	40	340	258	9
9	4339	86	4071	158	32	7	236	180	5
10	4435	378	3990	453	38	28	407	420	9
11	4359	93	4198	156	58	14	103	185	3
12	4359	93	3824	257	26	8	509	259	11

* In Table 20, page 87, are shown the distribution of the subjects among the age groups; the distribution of the five-day balance periods among the subjects; and, the average body weights and recumbent lengths of the subjects in each age group during the balance periods of study.

† The standard deviation (SD) was obtained with the formula: $\sqrt{\frac{\Sigma x^2 - N\bar{x}^2}{N-1}}$ in which N is the number of values, Σx^2 is the sum of the squares of the values, and $\bar{x}^2$ is the square of the mean of all the values.

† Retention is calculated as "Intake minus Outgo."

Of the mean daily chlorine intake of 3596 ± 460 mg. (101.4 meq.), for the 29 children, 277 ± 241 mg. (7.8 meq.), or eight per cent, was retained (Tables 86–88). This average retention value includes that lost through the skin. Because chlorine has such an important physiological role and exists so generally through the body, it is to be expected that it would be excreted in large measure through the kidneys. Ninety-one per cent of the mean chlorine intake was eliminated through the urinary tract, a mean excretion of 3265 ± 467 mg. (92.1 meq.) of chlorine daily. A small but definite quantity of chlorine was found in the stools, averaging one per cent of the intake, and amounting to 54 ± 33 mg. (1.5 meq.), per day.

TABLE 88

DISTRIBUTION OF CHLORINE EXCRETION BETWEEN URINE AND FECES IN 593 CHLORINE BALANCES FOR 29 CHILDREN

Age group	EXCRETION OF CHLORINE INTAKE		TOTAL DAILY EXCRETION IN URINE AND FECES		
	Urine	Feces	Mean	Urine	Feces
year	per cent	per cent	mg.	per cent	per cent
4	87	2	2997	98	2
5	90	2	3072	98	2
6	92	2	3226	98	2
8	89	2	3486	98	2
9	94	1	4103	99	1
10	90	1	4028	99	1
11	96	1	4256	99	1
12	88	1	3850	99	1

POSITIVE AND NEGATIVE MINERALS IN ELECTRONEUTRALITY AND THE FORMATION OF TISSUE

The alkaline or acid ash value of food differs from the reaction of the food ingested. As food is burned in the body, the organic anions, such as citrates, acetates and malates, become oxidized, releasing positive minerals and sulfur and phosphorus are oxidized to sulfates and phosphates. Although the anion values of the sulfur and phosphorus retained in the body may be debated, evidence indicates that the average valence of phosphorus in the human body may be considered as 1.8; of sulfur, 2.[312]

During normal growth there is an accumulation of both positive and negative minerals in new tissues, the total amount depending upon the intensity of growth. The relationship between the gross amounts of positive and negative minerals retained is determined by the relative demands for materials with which to construct hard or soft tissues. Hard tissue synthesis produces a demand for positive minerals; soft tissue construction requires a preponderance of negative minerals; therefore, increased retentions of positive minerals in relation to negative minerals indicate an impetus to skeletal construction and increased retention of negative minerals in relation to positive minerals evidences stimulation of soft tissue accretion.

The diets given the children in our studies contained an average of 463 meq. of positive and negative minerals per day. The diets provided from 427 to 600 meq. of "total minerals" (positive plus negative) to compensate for variation in the body needs of the children of different ages. Urine excretion paralleled the intake, ranging from 294 to 423 meq. daily. The largest portion of the mineral intake was used in maintaining electroneutrality in the body, for, of the average intake of 463 meq. for all the children, an average of 325 meq. per day was excreted in the urine; an average of 87 meq. was excreted through the feces; and, 51 meq. was retained. There was always a retention of total minerals, which is one of the best criteria that growth and development are taking place. On the basis of body weight the children were given an average of 21 meq. of total minerals per kilogram of body weight per day and retained 2.2 meq. At four years, from an average of 23 meq. of total minerals per day, an average of 3.2 meq. was retained, while at 12 years an average of 1.6 meq. per kilogram per day was retained from an intake of 14 meq.

Electropositive Minerals

The electropositive minerals (calcium, magnesium, sodium and potassium) are especially important in growth because of the role that calcium and magnesium play in skeletal formation, in

TABLE 89

AVERAGES OF 593 AVERAGE DAILY "POSITIVE MINERALS" BALANCES FOR 29 CHILDREN*

Values in milliequivalents

Age group	Intake		Outgo				Retention‡		
			Urine		Feces				
year	mean	SD†	mean	SD†	mean	SD†	mean	SD†	per cent intake
4	223.4	14.8	141.7	20.0	48.6	7.7	33.1	22.2	15
5	223.9	33.3	141.5	13.7	55.0	11.8	27.4	21.0	12
6	226.0	28.1	149.6	14.5	54.4	11.3	22.0	18.1	10
8	265.5	30.9	166.5	23.8	66.8	15.3	32.2	19.8	12
9	284.5	5.0	200.0	9.4	54.9	6.7	29.6	8.3	11
10	311.0	49.6	192.7	25.8	64.5	20.4	53.8	37.6	17
11	292.6	2.8	203.5	6.2	62.2	4.6	26.9	7.8	9
12	292.6	2.8	199.1	10.2	53.0	5.1	40.5	12.4	14

* In Table 20, page 87, are shown the distribution of the subjects among the age groups; the distribution of the five-day balance periods among the subjects; and, the average body weights and recumbent lengths of the subjects in each age group during the balance periods of study.

† The standard deviation (SD) was obtained with the formula: $\sqrt{\frac{\Sigma x^2 - N\bar{x}^2}{N-1}}$ in which N is the number of values, Σx^2 is the sum of the squares of the values, and $\bar{x}^2$ is the square of the mean of all the values.

‡ Retention is calculated as "Intake minus Outgo."

TABLE 90

AVERAGES OF 593 AVERAGE DAILY "POSITIVE MINERALS" BALANCES FOR 29 CHILDREN

Values in milliequivalents per kilogram of body weight

Age group	Intake		Outgo				Retention	
			Urine		Feces			
year	mean	SD	mean	SD	mean	SD	mean	SD
4	12.13	0.43	7.72	1.24	2.65	0.44	1.76	1.11
5	11.93	1.50	7.55	0.69	2.94	0.62	1.44	1.04
6	10.34	1.03	6.86	0.63	2.49	0.51	0.99	0.80
8	10.17	1.19	6.37	0.84	2.57	0.67	1.23	0.79
9	9.96	0.44	7.00	0.38	1.92	0.28	1.04	0.30
10	9.35	1.29	5.82	0.84	1.93	0.56	1.60	1.07
11	8.18	0.31	5.69	0.19	1.74	0.15	0.75	0.23
12	7.00	0.19	4.76	0.16	1.27	0.08	0.97	0.29

nervous and muscular activity and blood coagulation. Sodium and potassium contribute to the control of the body water, and, as well, participate in other structural and functional activities. The average daily positive mineral value of the diets of 29 children during 593 balance periods was 242 ± 40 meq., of which only 29 ± 22 meq. was retained. The average daily positive mineral content of the feces was 57 ± 14 meq., 57 per cent of which was from calcium alone (Tables 89–91).

TABLE 91

DISTRIBUTION OF "POSITIVE MINERALS" EXCRETION BETWEEN URINE AND FECES IN 593 "POSITIVE MINERALS" BALANCES FOR 29 CHILDREN

Age group	EXCRETION OF "POSITIVE MINERAL" INTAKE		TOTAL DAILY EXCRETION IN URINE AND FECES		
	Urine	Feces	Mean	Urine	Feces
year	per cent	per cent	meq.	per cent	per cent
4	63	22	190.3	74	26
5	63	25	196.5	72	28
6	66	24	204.0	73	27
8	63	25	233.3	71	29
9	70	19	254.9	78	22
10	62	21	257.2	75	25
11	70	21	265.7	77	23
12	68	18	252.1	79	21

The average daily consumption of positive minerals per kilogram of body weight was 10.7 ± 1.6 meq. On the same basis, 6.9 ± 1.0 meq. were excreted in the urine, 2.5 ± 0.7 meq. were eliminated in the feces and 1.3 ± 0.9 meq. were retained. There was a difference in the positive mineral intake of 12.1 ± 0.4 meq. to 7.0 ± 0.2 meq. per kilogram of body weight for the age groups 4 and 12 years, accompanied by similar differences in the excretion and retention values (Table 90).

Electronegative Minerals

Of the electronegative minerals, phosphorus, sulfur and chlorine, phosphorus is used in the formation of both soft and hard tissue and extensively in metabolism in general. Sulfur, although

TABLE 92

AVERAGES OF 593 AVERAGE DAILY "NEGATIVE MINERALS" BALANCES FOR 29 CHILDREN*

Values in milliequivalents

Age group	Intake		Outgo				Retention‡		
			Urine		Feces				
year	mean	SD†	mean	SD†	mean	SD†	mean	SD†	per cent intake
4	203.7	16.0	152.7	14.8	24.5	4.7	26.5	20.6	13
5	203.1	29.6	154.5	15.6	27.8	7.8	20.8	17.2	10
6	205.8	22.6	159.8	11.4	29.4	7.4	16.6	14.9	8
8	240.6	25.1	183.8	24.9	32.4	7.0	24.4	19.5	10
9	268.1	2.1	212.6	7.6	33.9	5.1	21.6	8.2	8
10	288.8	42.2	206.3	22.0	39.2	6.4	43.3	37.3	15
11	272.5	5.1	219.9	4.4	36.8	2.5	15.8	7.1	6
12	272.5	5.1	211.9	6.9	32.3	2.7	28.3	7.2	10

* In Table 20, page 87, are shown the distribution of the subjects among the age groups; the distribution of the five-day balance periods among the subjects; and, the average body weights and recumbent lengths of the subjects in each age group during the balance periods of study.

† The standard deviation (SD) was obtained with the formula: $\sqrt{\frac{\Sigma x^2 - N\bar{x}^2}{N-1}}$ in which N is the number of values, Σx^2 is the sum of the squares of the values, and $\bar{x}^2$ is the square of the mean of all the values.

‡ Retention is calculated as "Intake minus Outgo."

TABLE 93

DISTRIBUTION OF "NEGATIVE MINERALS" EXCRETION BETWEEN URINE AND FECES IN 593 "NEGATIVE MINERALS" BALANCES FOR 29 CHILDREN

Age group	EXCRETION OF "NEGATIVE MINERAL" INTAKE		TOTAL DAILY EXCRETION IN URINE AND FECES		
	Urine	Feces	Mean	Urine	Feces
year	per cent	per cent	meq.	per cent	per cent
4	75	12	177.2	86	14
5	76	14	182.3	85	15
6	78	14	189.2	84	16
8	76	14	216.2	85	15
9	79	13	246.5	86	14
10	71	14	245.5	84	16
11	81	13	256.7	86	14
12	78	12	244.2	87	13

retained in comparatively small quantities, is essential in soft tissue metabolism and in the formation of the organic matrix of the bone. Chlorine plays an essential role in water metabolism.

The average total negative mineral intake for the group of children was 221 ± 37 meq., ranging from 203 to 289 meq. for age groups of 4 to 12 years. The kidneys excreted 169 ± 26 meq., in contrast to 30 ± 8 meq. in the feces. The average daily retention was 22 ± 20 meq. (Tables 92–94). There was little relationship between intake and retention, in the different age groups. On the basis of body weight an average of 9.8 ± 1.4 meq. were consumed, and only 1.0 ± 0.8 meq. were retained per kilogram of body weight, daily (Table 94). Paralleling the smaller intake per kilogram of body weight per day with increased age, there were lower retentions, ranging from 1.4 ± 1.0 to 0.7 ± 0.2 meq. per kilogram body weight, daily.

TABLE 94

AVERAGES OF 593 AVERAGE DAILY "NEGATIVE MINERALS" BALANCES FOR 29 CHILDREN

Values in milliequivalents per kilogram of body weight

Age group	Intake		Outgo				Retention	
			Urine		Feces			
year	mean	SD	mean	SD	mean	SD	mean	SD
4	11.04	0.57	8.31	0.94	1.33	0.25	1.40	1.02
5	10.82	1.34	8.24	0.64	1.48	0.39	1.10	0.89
6	9.42	0.84	7.32	0.57	1.35	0.35	0.75	0.66
8	9.22	0.99	7.04	0.91	1.24	0.28	0.94	0.78
9	9.39	0.34	7.44	0.36	1.19	0.15	0.76	0.29
10	8.68	1.11	6.22	0.72	1.18	0.17	1.28	1.07
11	7.62	0.28	6.15	0.22	1.03	0.05	0.44	0.20
12	6.52	0.22	5.07	0.33	0.77	0.07	0.68	0.16

Excess Positive Minerals

If skeletal growth is to take place in a normal fashion there must be retained from the dietary an excess of total positive minerals over total negative minerals, since the bony tissue is predominantly composed of calcium, one of the constituents of the positive group. The percentage distribution of positive and negative minerals in the intake, outgo and retentions of our subjects is shown in Table 95. The average alkaline ash value of the dietaries given the children was 21 ± 7 meq. per day (Table 96). There was an average daily retention of 7 ± 12 meq. of cations in excess of anions, daily, while the average fecal cation value was 27 ± 9 meq.

TABLE 95

PERCENTAGE DISTRIBUTION OF POSITIVE AND NEGATIVE MINERALS BASED ON AVERAGES OF 593 BALANCES ON 29 CHILDREN AGED 4 TO 12 YEARS

	Intake	Outgo			Retention
		Urine	Feces	Total	
TOTAL POSITIVE					
Calcium	19	3	57	17	31
Magnesium	10	5	23	10	13
Potassium	29	36	17	31	19
Sodium	42	56	3	42	37
TOTAL NEGATIVE					
Phosphorus	33	24	76	31	46
Chlorine	46	54	5	47	35
Sulfur	21	22	19	22	19
TOTAL MINERALS					
Calcium	10	2	37	9	18
Magnesium	5	2	15	5	8
Potassium	15	17	11	16	10
Sodium	22	27	2	22	21
Phosphorus	16	12	26	15	20
Chlorine	22	29	2	23	15
Sulfur	10	11	7	10	8

On the basis of body weight, the average daily alkaline-ash value of the dietary ranged from 1.09 ± 0.33 meq. at the four-year-level to 0.48 ± .13 meq. at 12 years, with an average of 0.95 ± 0.35 meq. (Table 97). With the greater age and lower intake per kilogram there was a decrease in the excess of anions in the urine (0.59 ± 0.67 meq. to 0.31 ± 0.17 meq.; mean, 0.57 ± 0.45 meq.); and, of the excess positive minerals in the feces (1.32 ± .33 to 0.50 ± .04. meq.; mean 1.22 ± 0.44 meq.). The retentions of excess cations were similar throughout the age groups, averaging

0.30 meq. per kilogram of body weight per day, in spite of the fact the degree of variability within which the individual determinations ranged was large (SD 0.52). This similarity demonstrates that the type of growth taking place in these children was similar, and that their increase in size was of structural and functional character rather than of fatty tissue and water.

TABLE 96

EXCESS OF POSITIVE OR NEGATIVE MINERALS IN 593 AVERAGE DAILY BALANCES FOR 29 CHILDREN*

Values in milliequivalents

Age group	Intake		Outgo				Retention‡	
			Urine		Feces			
year	mean	SD†	mean	SD†	mean	SD†	mean	SD†
4	19.7^+	5.6	11.0^-	12.4	24.1^+	5.6	6.6^+	13.5
5	20.8^+	7.2	13.0^-	8.6	27.2^+	7.7	6.6^+	11.9
6	20.2^+	6.3	10.2^-	9.4	25.0^+	7.0	5.4^+	10.4
8	24.9^+	8.8	17.3^-	8.8	34.4^+	10.8	7.8^+	11.8
9	16.4^+	5.5	12.6^-	3.8	21.0^+	3.6	8.0^+	8.5
10	22.2^+	9.2	13.6^-	11.8	25.3^+	15.8	10.5^+	12.1
11	20.1^+	5.6	16.4^-	4.0	25.4^+	2.6	11.1^+	8.6
12	20.1^+	5.6	12.8^-	7.3	20.7^+	3.0	12.2^+	11.7

* In Table 20, page 87, are shown the distribution of the subjects among the age groups; the distribution of the five-day balance periods among the subjects; and, the average body weights and recumbent lengths of the subjects in each age group during the balance periods of study.

† The standard deviation (SD) was obtained with the formula: $\sqrt{\frac{\Sigma x^2 - N\bar{x}^2}{N-1}}$ in which N is the number of values, Σx^2 is the sum of the squares of the values, and $\bar{x}^2$ is the square of the mean of all the values.

‡ Retention is calculated as "Intake minus Outgo."

TABLE 97

EXCESS OF POSITIVE OR NEGATIVE MINERALS IN 593 AVERAGE DAILY BALANCES FOR 29 CHILDREN

Values in milliequivalents per kilogram of body weight

Age group	Intake		Outgo				Retention	
			Urine		Feces			
year	mean	SD	mean	SD	mean	SD	mean	SD
4	1.09^+	0.33	0.59^-	0.67	1.32^+	0.33	0.36^+	0.72
5	1.11^+	0.38	0.69^-	0.45	1.46^+	0.43	0.34^+	0.62
6	0.92^+	0.26	0.46^-	0.43	1.14^+	0.32	0.24^+	0.47
8	0.95^+	0.32	0.67^-	0.36	1.33^+	0.45	0.29^+	0.46
9	0.57^+	0.19	0.44^-	0.13	0.73^+	0.14	0.28^+	0.30
10	0.67^+	0.26	0.40^-	0.34	0.75^+	0.45	0.32^+	0.36
11	0.56^+	0.16	0.46^-	0.12	0.71^+	0.10	0.31^+	0.24
12	0.48^+	0.13	0.31^-	0.17	0.50^+	0.04	0.29^+	0.28

Formation of Soft Tissue

A positive nitrogen balance (excess of intake over outgo) may involve bodily storage of protein which becomes an integral part of the soft tissues (the muscles, parenchymatous organs and neural tissues in particular), of the cartilagenous or organic matrix of the bone or of the complex lipids composed of phospholipids (lecithins, cephalins, sphingomyelins), and cerebrosides. It is generally assumed, however, that the major portion of the nitrogen is deposited in the body as protein and that it carries with it the major portion of the stored sulfur (which arises mainly from the amino acids cystine and methionine) and from 30 to 40 per cent of the stored phosphorus.

Assuming that protein constitutes about one-fifth of the weight of the human body, the average five-year-old weighing 18 kilograms possesses around 3.6 kilograms of protein (equivalent to 576 grams of nitrogen). With the growth and development that takes place during the doubling of his age, his body at 10 years has reached a weight of 33 kilograms with a total protein concentration of 6.6 kilograms (equivalent to 1056 grams of nitrogen). Although we are accustomed to thinking of protein metabolism primarily in terms of nitrogen, the importance of nitrogen or nitrogen and sulfur in the form of specific essential amino acids in the protein molecule is assuming greater significance with the advent of the new and rapidly developing knowledge of the chemistry and physiology of the proteins and their constituent amino acids.

In the study of children the average nitrogen retention ranged from 5 to 8 per cent of the nitrogen intake while the sulfur retention lay between 6 and 17 per cent of the sulfur intake. The nitrogen to sulfur ratios have been calculated in the metabolic materials accumulated from 593 five-day balances on 29 children. Estimated in grams, the average nitrogen to sulfur ratio for childhood amounted to 14.2 (range 12.7 to 14.7) in the dietary, as compared to 15.0 (range 14.3 to 15.3) in the urine, 12.6 (range 10.2 to 13.8) in the feces and 9.1 (range 5.8 to 11.5) in the retention. Figure 52 shows the relation of nitrogen and sulfur retentions on the basis of the average daily amounts. There is a wide scatter among the nitrogen and sulfur retention figures of the various children on different occasions. There was disparity of results from individual studies conducted during different years; two groups indicate a definite relationship between nitrogen and sulfur retentions while another group showed no significant correlation, differences which suggest that level of sulfur intake, or other factors, may have played an influential role. Recent evidence indicates that the nutritive superiority of one type of protein over another would appear to be not so closely related to the amount of sulfur present in the protein or to the ratio of total sulfur to nitrogen[353] in the protein but rather, to be connected with the distribution of sulfur and nitrogen among the nutritionally essential amino acids.[9,19,335] The current specific demand for growth may exert its influence and be reflected in a characteristic type of retention. The low average N:S ratio of 9.1, compared to one of 15 to 16 in muscle tissue, suggests that cutaneous loss may be a factor in the sulfur retentions.

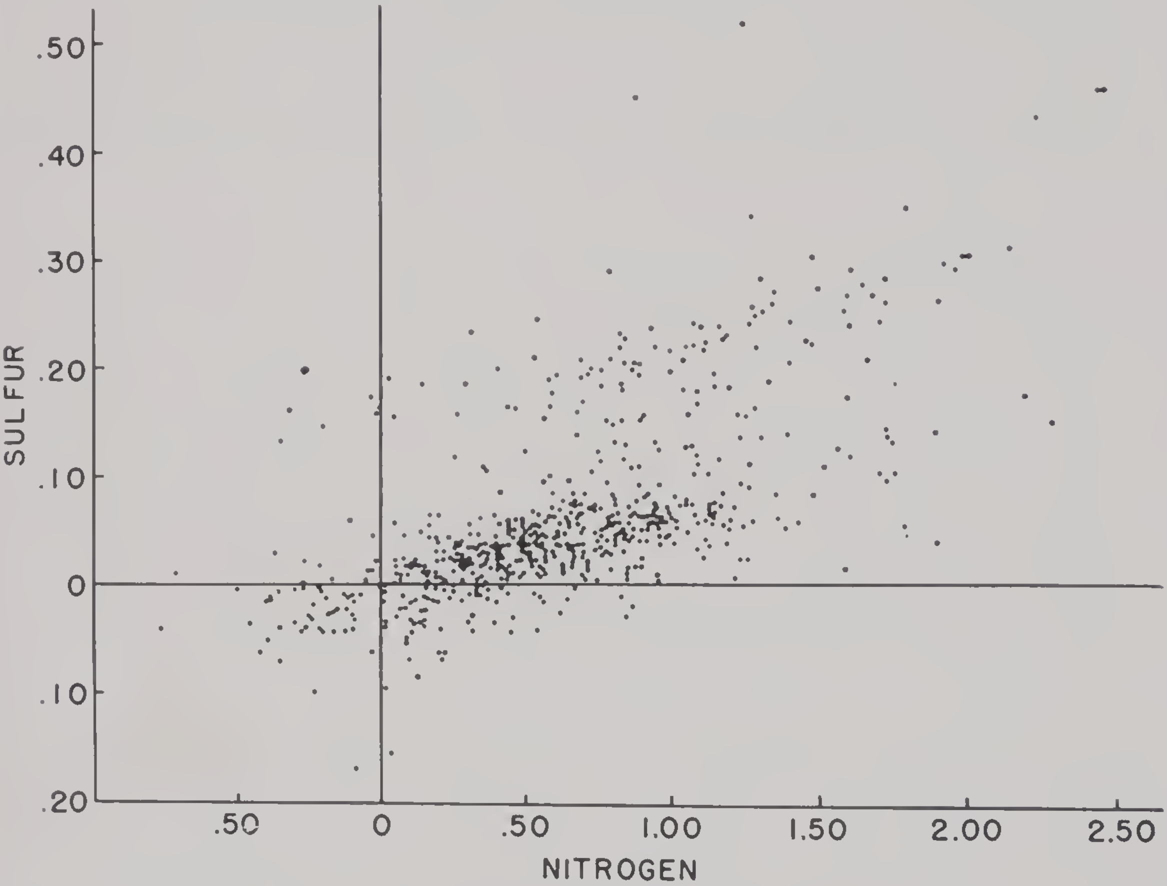


Figure 52. Average daily sulfur *vs.* nitrogen retentions.

It was found, through appropriate application of the analytical data available on the amino acid composition of the foods given, that of one gram of total sulfur of the daily dietary, methionine sulfur and cystine sulfur accounted for 0.51 gm. and 0.27 gm. spectively (Table 98). Most of the remaining 0.22 gm. of sulfur was probably in the form of sulfate, both alone or as chondrotin sulfuric acid. Of the total methionine plus cystine sulfur of 0.78 gm. approximately 0.59 gm. and 0.19 gm. came from the animal and vegetable sources, respectively. Breaking the sulfur down into its component sulfur containing amino acids in the respective animal and vegetable proteins, cystine sulfur amounted to approximately 0.18 and 0.09 gm. and methionine sulfur amounted to 0.41 and 0.10 gm. (See **Amino acids,** p. 86). Since sulfur in the form of these amino acids is so important for tissue formation in growth, a generous supply of animal protein seems to be indicated since it offers a better quality and a larger quantity of the essential amino acid, methionine. Both cystine and methionine are constitutents of tissue protein. Methionine is required by the growing organism not only for building of methionine-containing body proteins, but also for a variety of intermediary metabolic processes. This amino acid recently has been found to function as a source of available methyl groups in relation to choline and choline synthesis[361] and in the formation of creatine.[27,360,362]

TABLE 98
ESTIMATED DISTRIBUTION OF SULFUR CONTAINING AMINO ACIDS IN THE DIETARY

	Animal protein	Vegetable protein	Total dietary protein
	gm./day	gm./day	gm./day
Total sulfur			1.00
Cystine sulfur	0.17	0.08	0.25
Methionine sulfur	0.40	0.11	0.51
Cystine plus methionine sulfur	0.57	0.19	0.76

The phosphorus stored in the body participates largely in the maintenance and formation of skeletal tissue (about 70 per cent) and of soft tissue (about 30 per cent). In addition, phosphorus plays an important and diverse role in the metabolism of fats, carbohydrates, and minerals, and in the maintenance of the electroneutrality of the body. Because of the importance of nitrogen and phosphorus in numerous organic compounds associated with muscle, glandular and fluid tissue formation and maintenance in growth, nitrogen to phosphorus retention ratios are of especial significance. Estimated in grams per day, the mean nitrogen to phosphorus ratio was 8.5 (range 7.6 to 9.0) in the food intake, 13.0 (range 11.3 to 14.6) in the urine, 2.8 (range 2.6 to 3.6) in the feces and 3.6 (range 2.9 to 7.3) in the retention.

Potassium is primarily a cellular constituent, whereas sodium is largely present in the extracellular fluids. The fact that the body contains less potassium than sodium lends credence to the belief that the larger the potassium to sodium retention ratios indicate that soft tissue is being formed faster than hard. If this assumption is correct the retentions of nitrogen and potassium should also show some relationship to one another, since nitrogen retention indicates the formation of protein tissue. On the basis of grams the average nitrogen to potassium ratio in the food was 3.8 (range 3.7 to 4.0), in the urine 4.1 (range 3.6 to 4.2), in the feces 3.0 (range 2.4 to 5.4) and the retention 3.0 (range 2.3 to 6.5).

The construction of new muscle, glandular and neural tissue involves particularly the storage of nitrogen, sulfur, phosphorus, potassium, sodium and chloride. Nitrogen, sulfur and a portion of the phosphorus are used primarily as structural components in the protein-lipid complex of the cell; potassium, sodium, chlorine and some of the phosphorus serve a functional purpose in the maintenance of the electroneutrality of the tissues with an equitable distribution of intracellular and extracellular fluid; nitrogen, phosphorus and possibly sulfur in the lipid components. The degree of storage of any one or all of these elements will be influenced by the food intake and the current nutritive needs of the individual.

Although it is known that the retention values include the cumulative errors of the metabolic balance procedures and analytical technique, especially insofar as potassium and sulfur (some amounts may be lost through the skin) are concerned, the wider range of variabilities observed in the retention values in comparison to those in the other metabolic materials seem to have significance beyond those which may be assigned to technical errors. The wide range found in the nitrogen to sulfur retention ratio, the nitrogen to phosphorus ratio and the nitrogen to potassium ratio indicate the variability of the nutritive needs of the body and the body's ability to choose those elements in the quantities which best meet the current structural and functional requirements.

Formation of Skeletal Tissue

It is generally assumed that of the mineral elements stored in body construction and maturation, approximately 98 per cent of the calcium, 70 per cent of the magnesium and 70 per cent of the phosphorus become a part of the skeletal structure while the remainder may be used either as structural components of soft and fluid tissues or as functional components in intermediary metabolism. General body growth can be measured in terms of chemical units. A storage of both positive (calcium, magnesium, sodium and potassium) and negative (phosphorus, sulfur and chlorine) minerals accompanied by nitrogen demonstrate that muscles, glandular, nervous, fluid and bony tissues are either enlarging or developing, or both may be taking place.

Calcium makes up a large proportion of the mineral cations stored and is the major constituent of bone. Bone construction therefore is indicated by either a storage of calcium alone, of total mineral cations (calcium, magnesium, sodium, potassium) or of an excess of mineral cations in relation to anions (phosphorus, sulfur, chlorine).

The average daily calcium to phosphorus ratio in the dietary of our children was 0.74 (range 0.63 to 0.80) which resulted in an average ratio in the urine of 0.14 (range 0.08 to 0.17), in the feces 1.64 (range 1.33 to 1.95) and in the retention 1.05 (range 0.88 to 1.33). The wide variations in the average calcium to phosphorus ratio in the retention demonstrate changing demands for the minerals laid down in the skeletal structure and in the soft muscular and glandular and nervous tissues. The average calcium to phosphorus retention ratio of under one indicates a rapid formation of soft tissues while a ratio of 1.30 indicates a relatively equal rate of formation of bone and soft tissue, according to Stearns.[328] Average values for groups of children obscure the significant trends that take place from time to time in the individual child. These individual trends will be the subject of discussion in the second volume.

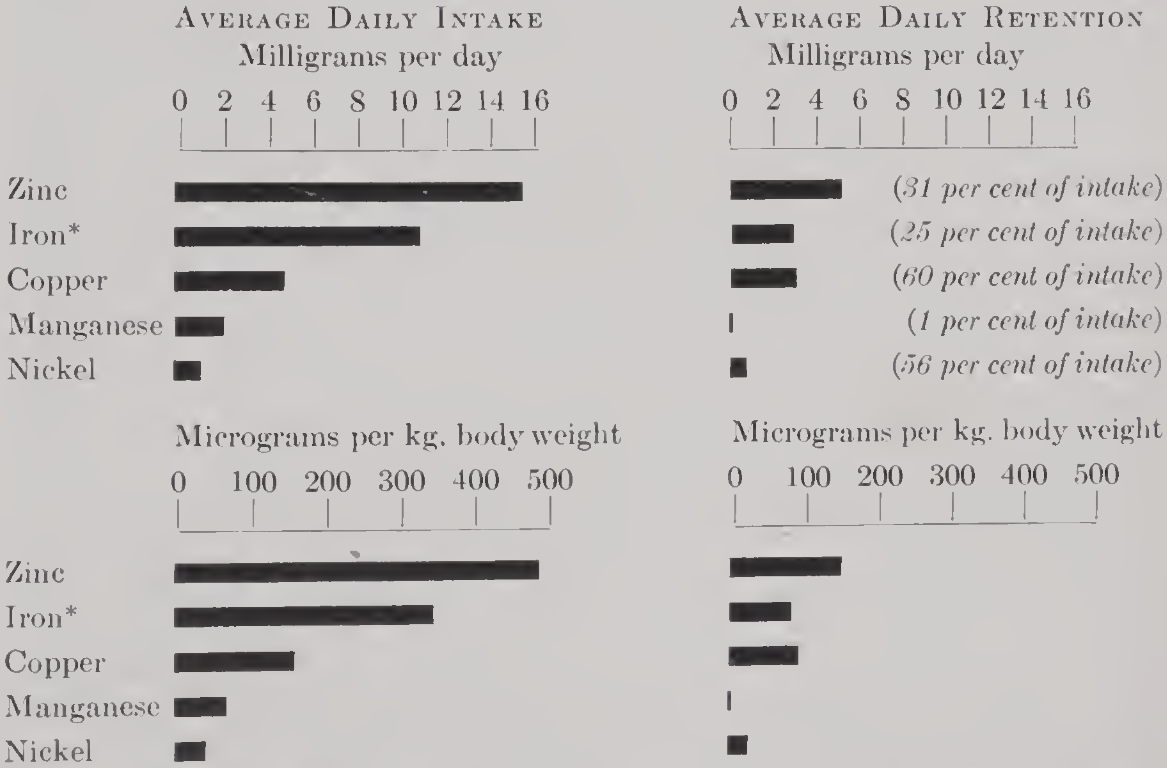
The homostatic characteristics of the human body are demonstrated through its great flexibility and dynamic qualities. Although endowed by heredity with a given capacity for growth and development the body's attainment in this respect is modified from day to day by its external and internal environment. Change and flexibility are essential characteristics of growth if the organism is to survive and meet with equanimity the necessary increasing structure and physiological function imposed by heredity in spite of some of the eccentricities of environment and mankind. Variability in chemical growth is therefore to be expected.

The type of chemical growth taking place at any one time will depend upon age or state of growth, the quantity and proportions of nutriments in the dietary, the internal environment and nutritive stage, the external environment and physical activity. Supplied with an adequate diet the body chooses what it needs. If depleted tissues and unfilled stores exist, there will be the tendency to replenish these when an opportunity arises. Growth and replenishment of depleted tissues and body stores may occur simultaneously but the metabolic balance method of study does not permit a differentiation of the various functions. Hidden hunger may actually exist in a run-about child and escape our present methods of detection. If one wishes to study the chemistry of healthy growth, the first prerequisite is the preparation of the child by subjecting him to a well regulated life within an environment judged by competent authorities to be satisfactory for his particular physical, mental and social progress, to furnish him with a dietary composed of adequate quantities of the known essential nutrients and in such proportions to one another as to permit satisfactory utilization by the body for whatever needs exist, and to extend the period of preparation over sufficient time to permit replacement or construction of depleted or inadequate tissues and body surpluses. Satisfactory preparation may be accomplished within a few weeks for one child in contrast to several months for another, depending upon the extent of depletion, maladjustment or hidden hunger characterizing the child and the rapidity and completeness of his response to the standardization procedures. Not until nutritional stability of the child becomes a reality within a well-ordered environment where experimental errors in techniques and control have been reduced to a minimum is it possible to observe with some degree of satisfaction the vicarious activities and trends accompanying the growth and nutrition processes. Indeed, under prolonged observations the metabolic balance procedure, accompanied by the many related and diverse physiological and chemical observations recorded in this volume, not only gives information on the total body growth but differentiates some of the subtle chemicophysiological adjustments and trends that take place in the growth of soft and skeletal tissues.

COPPER, MANGANESE, NICKEL, ZINC AND IRON

For many years the prevalence of various types of anemia and the knowledge that iron is a major component of hemoglobin has stimulated intensive studies of iron metabolism. In recent years a growing awareness of the possible requirements for heavy metals in cell metabolism and enzymatic processes has encouraged investigation of the nutritional roles of these substances. Development of the tracer element method of study and improvements in analytical methods have provided the means for broadening our knowledge in this direction. Indications have been presented that copper, cobalt, zinc and manganese may be essential to normal cell function.[114] Copper and zinc are to be found in the human brain and traces of copper and cobalt assist iron in maintaining a steady rate of hemoglobin formation.

To illustrate the relative amounts of these elements, and iron, which were ingested and retained during the studies of nutrition and chemical growth in childhood, we have charted quantitative intakes and retentions, daily and per kilogram of body weight, in Figure 53.



* Only the iron balances corresponding to those obtained for the other heavy metals were used in deriving this figure.

Figure 53. Intake and retention of heavy metals.

We have been able to obtain copper, manganese, nickel and zinc balances with some of the children who participated in the investigation of nutrition and chemical growth in childhood. During the study of seven children, 8 to 12 years old, over 55 consecutive days, 254 balances for these four, so-called "trace" elements were determined. The distribution of the balances, according to age of the children, is shown in Table 99, with the corresponding weights and recumbent lengths. The average daily balances for the four elements are given in Table 100 and the average distribution of the elements in the outgo in Table 101. It should be recognized that the classic method of balance study does not reveal what portion of the retention value represents possible cutaneous loss. An investigation of the loss of heavy metals by this route should be a fruitful field of endeavor, since these elements are known to be associated with integumental tissues.

TABLE 99

AVERAGE LENGTHS AND WEIGHTS OF SEVEN CHILDREN WHO WERE SUBJECTS OF METABOLIC STUDY DURING 254 BALANCE PERIODS

Age group	Subjects	FIVE-DAY BALANCES DETERMINED				Average body weight	Average recumbent length
		Copper	Manganese	Nickel	Zinc		
year	number	number	number	number	number	kg.	cm.
8	1	11	11	10	11	27.08	132.2
9	2	21	22	11	21	28.58	136.0
10	2	20	20	9	21	32.72	142.3
11	1	10	10	7	10	35.75	138.3
12	1	10	9	—	10	41.84	159.1
Total	7	72	72	37	73		

Copper

The copper concentration in the liver of infants is many times greater than in that organ of the adult (24 mg. and 4 mg., respectively, per kilogram of fresh tissues).[242,274] Apparently, the young store surpluses of iron and copper in the liver during intrauterine life, to tide them over the first 12 months of extrauterine existence. This is shown, not only by analyses of morbid tissues, but also by studies of the blood hemoglobin of healthy infants (pp. 29, 34). Copper has been shown to be of fundamental importance in the formation of hemoglobin in red-blooded animals.[76,124,125] These findings have found application in the clinic. Experiments indicate that copper has no influence on iron assimilation but does function in the conversion of iron into hemoglobin after it has been absorbed. Elvehjem[50,295] demonstrated that feeding copper, or copper and iron, increases the oxidase content of the liver of anemic rats; iron alone had no effect. More recently the importance of copper and iron on the catalase activity of the liver, kidney and blood has been reported.[296]

TABLE 100

AVERAGES OF AVERAGE DAILY BALANCES OF COPPER, MANGANESE, NICKEL, AND ZINC FOR CHILDREN*

Averages per day (mg.)	Copper		Manganese		Nickel		Zinc	
	mean	SD†	mean	SD†	mean	SD†	mean	SD†
Intake	5.03	0.69	2.16	0.14	1.12	0.32	15.92	1.11
Urine	0.34	0.16	0.01	0.02	0.01	0.01	0.48	0.22
Feces	1.68	0.39	2.13	0.18	0.48	0.08	10.52	2.78
Retention	3.01	0.80	0.02	0.22	0.63	0.31	4.92	2.94
Averages per kilogram of body weight (micrograms)								
Intake	158	27	68.20	9.61	36.92	10.84	500	65
Urine	11	5	0.30	0.77	0.30	0.32	15	8
Feces	53	14	67.26	9.81	15.87	3.24	331	90
Retention	94	26	0.64	6.64	20.75	10.38	154	88

* See Table 99 for distribution of subjects and number of balances determined.

† The standard deviation (SD) was obtained with the formula: $\sqrt{\frac{\Sigma x^2 - N\bar{x}^2}{N-1}}$ in which N is the number of values, Σx^2 is the sum of the squares of the values, and $\bar{x}^2$ is the square of the mean of all the values.

From records of hemoglobin content and cellular structure the children of our studies were supplied with copper in assimilable form and in adequate amounts to fulfill the needs of the hematopoietic system (See Chapter IX). The average copper balance for seven children, ages 8 to 12 years is shown in Table 100. Of an average intake of 5.03 mg. of copper, daily, 1.68 mg. were excreted by the colon and 0.34 mg. by the kidneys. The average daily retention per child during the 360 experimental days was 3.01 mg. per day. The average daily copper intake was less than half the iron intake, 5.03 mg. and 11.72 mg., respectively, but the retentions of the two metals were essentially the same (2.92 mg. iron; 3.01 mg. copper). For both elements, the greatest excretion occurred in the feces, but copper excretion in urine was approximately twice that of iron. We have no knowledge of losses through the skin.

The average daily copper intake and retention amounted to 158 and 94 micrograms per kilogram of body weight, respectively. The ratio of copper to iron was 0.43 in the dietary and 1.03 in the retention. The copper intake of our children happened not to greatly exceed their retention, but, judging from hemoglobin formation and cellular structure, there were sufficient amounts to meet the needs of the hematopoietic system and growth and health were satisfactory.

TABLE 101

DISTRIBUTION OF EXCRETION OF HEAVY METALS BETWEEN URINE AND FECES IN 254 BALANCES FOR SEVEN CHILDREN

	EXCRETION OF INTAKE		AVERAGE DAILY EXCRETION IN URINE AND FECES		
	Urine	Feces	Urine + Feces	Urine	Feces
	per cent	per cent	mg.	per cent	per cent
Copper	6.8	33.4	2.02	16.8	83.2
Manganese	0.5	98.6	2.14	0.5	99.5
Nickel	0.9	42.9	0.49	2.0	98.0
Zinc	3.0	66.1	11.00	4.4	95.6

Zinc

Zinc has long been classified as one of the so-called "trace elements" in nutrition. Actually, zinc is universally distributed in plant and animal tissues and is stored in appreciable quantities in the liver and kidneys. Sporadic investigations have shown that zinc normally is a constituent of the red blood cell, and because of this fact it has been considered in relation to hemoglobin formation. Within the past five years there has been renewed interest in the nutritional significance of zinc and more intensive tests have been contrived to determine its specific physiologic role. These studies have been concerned with the occurrence and form of zinc in blood and urine, in health and disease, especially with its association with carbonic anhydrase in the red blood cell[151] and the zinc-prophyrin compound excreted in the urine;[171] with its influence upon endogenous metabolism in relation to protein and carbohydrate; and, with its association with particular enzymatic and hormonal activities.

The daily dietary of the seven children studied by this Laboratory furnished an average of 15.92 ± 1.11 mg. of zinc (Table 100). Of this amount, 0.48 ± 0.22 and 10.52 ± 2.78 mg. were excreted in the urine and feces, respectively; 4.92 ± 2.94 mg. were retained. The feces contained 96 per cent of the zinc excreted. On the basis of body weight, the children's intakes averaged 500 ± 65 micrograms, and their retentions 154 ± 88 micrograms per kilogram per day, indicating a relatively high zinc requirement for children and contradicting classification of zinc as a trace element.

We have found a record of only one other zinc balance study on children,[298] made on younger subjects who consumed smaller quantities of food and retained less zinc. The recent discoveries[173, 174] that carbonic anhydrase contains, consistently, at least 0.3 per cent zinc, and that all of the zinc in the red blood cell is present in this form, accounts for part of the zinc retained by our subjects. Apparently, the element has other functions, associated with enzymatic and hormonal activities in other tissues. Whether the zinc allowance given these children was adequate and in the optimal proportions to other elements in the diets, such as copper and iron, must await future investigations.

Manganese

Manganese, widespread in occurrence in the tissues of animals, has been demonstrated to be necessary for satisfactory physiological functioning of the bodies of animals. There is abundant evidence that manganese is necessary for normal reproduction in the rat and for the prevention of certain avian bone disorders. Recent investigations have linked manganese with oxidative enzymes. Little is known concerning the role of manganese in human nutrition, although it has been associated with blood formation and has been used clinically for treatment of secondary anemia.

The average daily intake and retention of manganese by our subjects were 2.16 ± 0.14 and 0.02 ± 0.22 mg., respectively. Excretion in urine was exceedingly small, an average of 0.01 ± 0.02 mg., 0.5 per cent of the intake. The major portion (99 per cent) of the intake was excreted through the bowel. Whatever the physiologic function of manganese in the body, it is needed in very minute quantities, for the average daily manganese intake and retention per kilogram of body weight were 68.20 ± 9.61 and 0.64 ± 6.64 micrograms, respectively.

The children observed in this investigation were growing at a satisfactory rate and were in the best of health, judged by growth records, blood analyses and metabolic studies. Whether a different level of manganese intake would have promoted better growth is a speculative question, for the only other metabolic balance study of manganese, with children, is one by Everson and Daniels.[90] From their study of preschool children they suggest that children's daily diets should contain daily 0.2 to 0.3 mg. per kilogram of body weight.

Nickel

Nickel has not been demonstrated to be an essential element in nutrition. At times, nickel has been associated with treatment of blood dyscrasias, as have copper, iron, manganese and zinc. We have had the opportunity of first recording nickel balances for children (Tables 100–101). The intake of nickel was very small, 1.12 mg. per day and was quite constant for all the balances determined, which is shown by the standard deviation of 0.32 mg. More than half of the nickel intake was retained, 0.63 ± 0.31 mg., 56 per cent of the intake. The feces contained 43 per cent (0.48 ± .08 mg.) of the intake and urine 1 per cent (.01 ± 0.01 mg.). The children ingested and retained 36.92 ± 10.84 and 20.75 ± 10.38 micrograms per kilogram of body weight per day, respectively, an amount of nickel greatly exceeding the amount of manganese (0.64 microgram) retained on a unit weight basis.

Iron

Iron balances obtained with 15 children during 463 balance periods are presented in Tables 102–105. The average daily intake for the group was 8.43 ± 1.32 mg. Elimination of iron occurred almost entirely *via* the intestine, averaging 7.39 mg. per day in contrast to an average of 0.18 mg. in urine. McCance and Widdowson[211,212,379] state that iron excretion in feces is no criterion of the body's utilization but is a reflection of its absorption, for the gastrointestinal tract is not able to regulate by excretion the amount of iron in the body." Our subjects' mean retention of iron was only 0.86 ± 4.23 mg. per day, 10 per cent of their intake. Although 8.43 mg. of iron per day is not an excessive intake, it falls within the recommendations of the Committee on Food and Nutrition of the National Research Council.

The average daily retention of iron per kilogram of body weight was 37 micrograms from an intake of 381 micrograms. These iron retentions are lower than the values recently obtained by Porter[271] on younger children.

We have made no studies on the availability of the iron in the

TABLE 102

AVERAGE LENGTHS AND WEIGHTS OF 15 CHILDREN WHO WERE SUBJECTS OF METABOLIC BALANCE STUDIES DURING 463 IRON BALANCE PERIODS

Age group	Subjects	Five-day balances determined	Average body weight	Average recumbent length
year	number	number	kg.	cm.
4	1	43	17.99	108.6
5	3	126	18.66	111.2
6	4	167	21.77	119.3
8	3	85	26.24	129.8
9	1	11	29.04	140.9
10	2	21	32.72	142.3
12	1	10	41.84	159.1

TABLE 103

AVERAGES OF 463 AVERAGE DAILY IRON BALANCES FOR 15 CHILDREN*

Values in milligrams

Age group	Intake		Outgo				Retention†		
			Urine		Feces				
year	mean	SD‡	mean	SD‡	mean	SD‡	mean	SD‡	per cent intake
4	7.95	0.56	0.11	0.13	6.96	3.35	0.88	3.22	11
5	7.75	0.47	0.18	0.24	6.95	5.31	0.62	5.21	8
6	8.17	0.52	0.19	0.20	7.79	4.72	0.19	4.73	2
8	8.53	0.32	0.21	0.29	6.83	1.62	1.49	1.57	18
9	11.16	2.25	0.07	0.03	8.61	0.95	2.48	2.07	22
10	11.80	2.24	0.21	0.24	8.47	1.68	3.12	2.50	26
12	12.18	2.06	0.07	0.03	9.12	1.25	2.99	2.50	24

* In Table 102, above, are shown the distribution of the subjects among the age groups; the distribution of the five-day balance periods among the subjects; and, the average body weights and recumbent lengths of the subjects in each age group during the balance periods of study.

† Retention is calculated as "Intake minus Outgo."

‡ The standard deviation (SD) was obtained with the formula: $\sqrt{\frac{\Sigma x^2 - N\bar{x}^2}{N-1}}$ in which N is the number of values, Σx^2 is the sum of the squares of the values, and $\bar{x}^2$ is the square of the mean of all the values.

food given to the children. Elvehjem, Hart, Sherman, and others[2,26,77,78,184,310,318] have reported that it is difficult to evaluate foods as a source of iron in the diet from their total iron content, because the availability of iron in foods may vary from less than 20 to over 90 per cent of the total iron content. Assuming that only 50 per cent of the total iron in the dietary was available, there would have been sufficient available iron allowed to meet the daily requirement of these children. Whether the iron reserves in the liver, the spleen or the bone marrow influenced these balances is not known. It is safe to surmise that these would be utilized to some extent before hemoglobin production would suffer.

TABLE 104

DISTRIBUTION OF IRON EXCRETION BETWEEN URINE AND FECES IN 463 AVERAGE DAILY IRON BALANCES FOR 15 CHILDREN

Age group	EXCRETION OF IRON INTAKE		AVERAGE DAILY EXCRETION IN URINE AND FECES		
	Urine	Feces	Urine + Feces	Urine	Feces
year	per cent	per cent	mg.	per cent	per cent
4	1	88	7.07	2	98
5	2	90	7.13	3	97
6	2	96	7.98	2	98
8	2	80	7.04	3	97
9	1	77	8.68	1	99
10	2	72	8.68	2	98
12	1	75	9.19	1	99

TABLE 105

AVERAGES OF 463 AVERAGE DAILY IRON BALANCES FOR 15 CHILDREN

Values in micrograms per kilogram of body weight

Age group	Intake		Outgo				Retention	
			Urine		Feces			
year	mean	SD	mean	SD	mean	SD	mean	SD
4	442	16	6	7	385	174	51	174
5	416	25	10	12	369	260	37	261
6	376	17	9	9	357	209	10	214
8	327	26	8	10	261	57	58	60
9	384	76	2	1	297	33	85	71
10	360	69	6	7	259	56	95	75
12	291	49	2	1	218	30	71	60

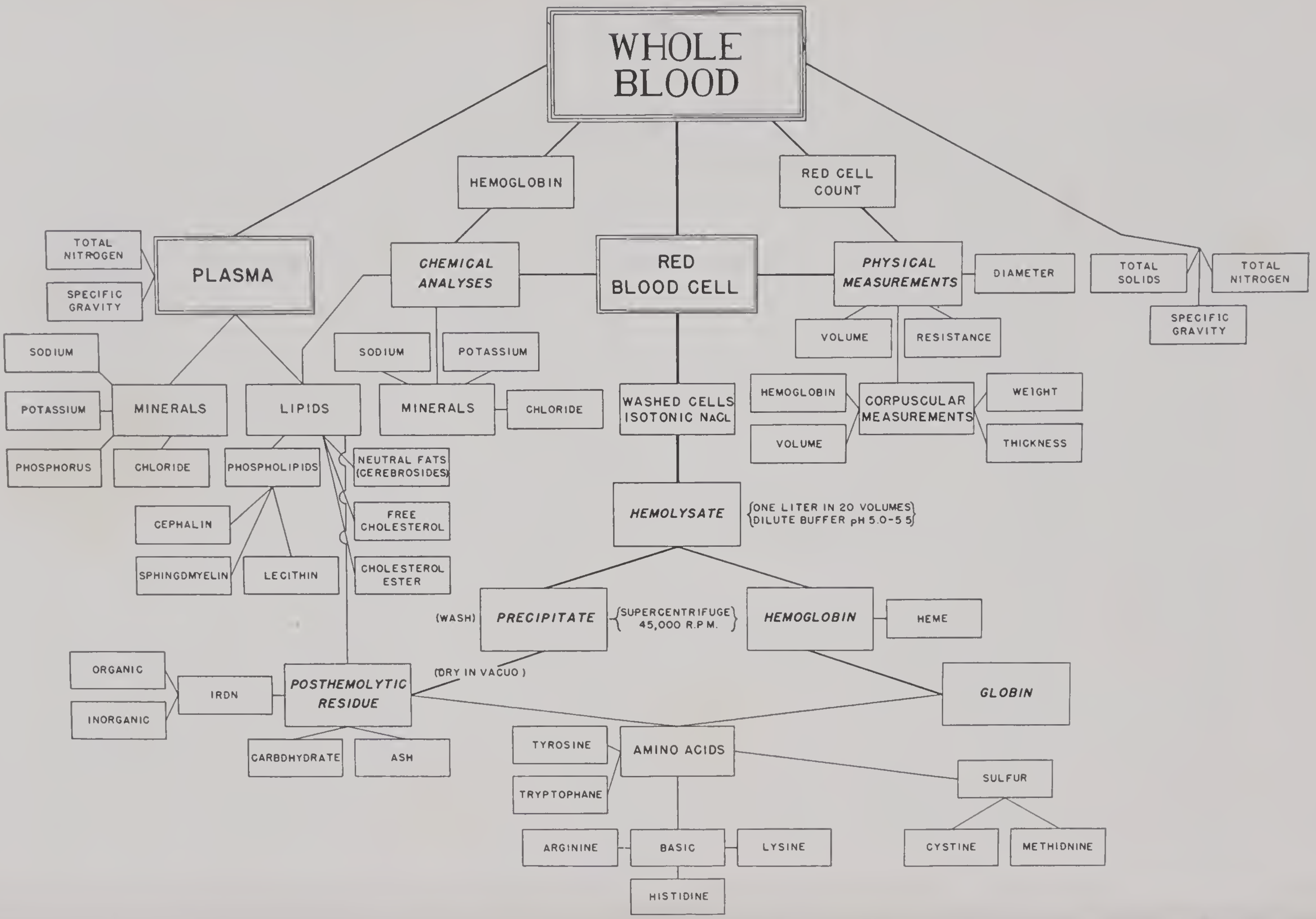


FIGURE 54. Integrated program of blood investigation carried on by the Research Laboratory of the Children's Fund of Michigan.

CHAPTER IX

HEMATOCHEMICAL STUDIES

With Special Emphasis on the Formed Elements

IT HAS been shown that there are variations in the hemoglobin and erythrocyte contents of the blood from infancy to maturity.[116,244,257,258] The following hematological observations have been made in this laboratory, using standardized techniques on healthy individuals and are recorded as a matter of reference even though there are few individuals in some of the groups.

As a part of the study of nutrition and chemical growth we have chosen to study the chemistry of the erythrocytes and other formed elements in the blood as a means of gaining information on the structure and nutrition of cells in general. Special emphasis has been given to the red blood cell because it exists free in a circulating medium which is in changing equilibrium with the surrounding tissues. The erythrocytes can be isolated and separated in sufficient quantities for thorough hematological and chemical study. An effort has been made, as will be shown on the following pages, to determine what proportions and which of the various components are combined structurally in the framework and covering (stroma), and which, if any, are loosely held or are mobile transport constituents within the cell. In view of the fact that physicochemical behavior of the red blood cell is dependent upon its chemical composition and surrounding medium a coordinated physical and chemical study is indicated.

Specific chemical constituents are essential in cellular structure and function. Impairment of physiological activity and efficiency may result from pathological changes which the corpuscle undergoes. Special emphasis in our investigation of cell composition and structure has included hematological observations, and protein, lipid and mineral analyses of formed elements of the blood, in health and in specific diseases. For instance, by studying the red blood cell during the course and treatment of certain blood dyscrasias it has been possible to observe the hematological and chemical changes that take place in the individual cells as they are influenced by immaturity or maturity, by increased or decreased hematological and metabolic activity, by inherent defects in structural characteristics and by environment. The information gained from the study of the chemical composition and structure of the red blood cells, white blood cells and platelets is of fundamental importance in cellular physiology and in nutrition and growth. As a matter of fact, much of the information is of special value in determining the health status of the body as a whole and of the blood forming organs in particular. It also indicates that the extension of these and other blood studies from the period of viability in infancy to maturity may be fruitful in the identification of the physiologic states which characterize the different stages of human development. The knowledge we have gained on the distribution of the minerals and lipids in the plasma and erythrocytes in the blood during childhood and the plasma, erythrocytes and stroma (posthemolytic residue) of normal human blood and of blood platelets is summarized in the following section.

Observations have been made on the allocation of lipids, electrolytes and protein in the erythrocytes, leucocytes, platelets and plasma of the blood during childhood and adulthood, during the course and treatment of different kinds of blood dycrasias, and upon bloods representative of different species of animals. Special effort has been made to identify in the formed elements of the blood those constituents which serve as an integral part of the cell framework and differentiate them from those components which may exist either more or less in a physiologically constant state or in a mobile fluctuating state. The structural components would be expected to possess a greater constancy and not be readily influenced by the changes accompanying growth and environmental conditions while the more mobile or metabolic constituents would probably reflect the nutritional status and certain environmental changes. The structure and function of the cells are dependent upon the presence of lipids, electrolytes and protein and their synergism. With our interest focused on nutrition and chemical growth, we have followed a somewhat uncharted course in the study of blood. Figure 54 diagrams the integrated blood program as it has developed.

It is known that certain constituents of the blood change during the course of specific pathological conditions and the determination of the concentrations of these in the blood or plasma may be used as a guide in the diagnosis and treatment of disease. Any investigation of the blood may be made just as extensive in scope as technical skill, time and money will permit.

In contrast to the study of disease, the quantitative evaluation of the more subtle physiological fluctuations accompanying growth is more difficult to define. Changing structure and function, as characterized by growth and development, may elicit one type of physiological response while maintaining equilibrium in the presence of diverse nutritive and environmental factors may elicit another type of reaction. It has been necessary to develop new methods and refine old ones, to permit numerous simultaneous observations on the same sample of blood, an enlargement of the scope of the investigation and an integration of all results.

Erythrocytes and Plasma

Pertinent information on the character and activity of the hematopoietic system can be obtained through hematological observations accompanied by certain physical measurements. These include determinations of cell volume, weight, diameter, thickness, specific gravity, water content, and fragility or resistance to hemolysis against saponin and hypotonic sodium chloride solutions.[267,268] Under normal conditions the erythrocyte of man has a specific biconcave shape which has been characterized as a compromise between a sphere and an infinitely thin disc. This peculiar shape seems to provide the greatest carrying capacity, with the least amount of structural material, while presenting the most favorable conditions for diffusion. The semipermeable membrane surface of the erythrocyte enclosing the hemoglobin, salts and other materials in solution permits it to function efficiently in an orderly manner.[4]

The shape and size of the red blood cell as expressed by volume, diameter, and thickness relationships play a significant role in its physicochemical behavior and therefore its physiological activity or efficiency, both of which are so important to nutritive success and efficiency in somatic structural growth and development. There are varying degrees of abnormalities arising in the borderline between health and disease in which the physical and chemical characteristics of the erythrocyte may have a bearing. Keeping in mind the degrees and kinds of change that may take place between the beginnings of impaired function and recognizable established disease it seems worth while to illustrate how the normal blood characteristics[80] may be changed under conditions of known blood dyscrasias. The following examples will serve to demonstrate the need for a careful detailed study of the blood in investigations of nutrition and chemical growth. The erythrocytes in hemolytic anemia are more spherical in contour and are characterized by increased fragility to hemolysis by saponin and hypotonic sodium chloride solutions whereas the cells of erythroblastic anemia are more discoidal than normal and are less vulnerable because of their increased resistance to hemolytic agents.[81] The macrocytic erythrocyte in pernicious anemia has slightly greater sphericity than the normal red blood cell, as shown by the diameter to thickness ratio and volume to thickness index, and demonstrates a slight increase of resistance in both beginning and complete hemolysis by hypotonic sodium chloride but a slightly greater fragility than normal in saponin solution.[383] However, the microcytic erythrocyte of hypochromic anemia is more discoidal in shape but seems to have a normal resistance to hemolytic agents.[81] Comparison of the surface area of the macrocytic erythrocyte of pernicious anemia, with its more expansive surface than the normal and an increased resistance in hypotonic sodium chloride, and the microcytic erythrocyte of hypochromic anemia, suggests a relationship between fragility and the corpuscular area.[383]

Although the physicochemical properties of the erythrocyte surface have been well defined by a variety of measurements, comprehensive information concerning the chemical nature of the erythrocyte membrane does not exist. In a series of correlated hematological, physical and chemical studies of the blood and stroma we have made an attempt to contribute further knowledge concerning the chemical nature of the erythrocyte membrane in different species, in both normal and pathological human and animal bloods.[7,16,82,85,86,385,386]

Samples of blood for chemical analysis and stroma preparation were removed by venipuncture. Determinations of red blood cell count, hemoglobin, hematocrit, diameter, specific gravity, and water content were made; from these, corpuscular volume, weight, thickness, and hemoglobin and water contents were calculated. The chemical data included mineral analyses (sodium, potassium, chloride,* calcium, and phosphorus) of the serum and red blood cells; the lipid distribution (free, and esterified cholesterol, total phospholipid, lecithin, cephalin, sphingomyelin, cerebrosides, and neutral fat) of the plasma and erythrocytes; and the amino acid and lipid partition of the stroma prepared from the erythrocytes. Practically all of the lipids of the erythrocyte are separated with the stroma. The different hematological and physical characteristics of the red blood cells obtained from various sources seem to be associated with the quantitative relationship of the protein-lipid complex of the cell structure or framework. The protein and lipid patterns of the red cell stroma are similar in the different species. In certain pathological conditions, however, the lipid pattern may undergo marked alterations.

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* Chlorine and chloride have been used interchangeably in referring to Cl in blood.

TABLE 106

HEMATOLOGICAL OBSERVATIONS ON NORMAL CHILDREN

	Mean	Standard Deviation
Erythrocytes		
Whole blood, millions per c.mm.	4.76	0.41
Per gram of cells $\times 10^{10}$	1.09*	0.11
Specific gravity	1.0886	
Water content, per cent weight	66	
Hemoglobin		
Whole blood, grams per 100 ml.	13	1
Individual cells, micromicrograms	28	3
Hematocrit, per cent	40	7
Corpuscular Measurements		
Volume, cμ	84	6
Weight, micromicrograms	91	10
Diameter, μ	7.2	0.9
Thickness, μ	2.1	0.1
Diameter to thickness ratio	3.4	0.3
Volume to thickness index	1.15	0.01
Resistance		
Hypotonic sodium chloride		
Hemolysis Beginning, per cent of NaCl	0.41	
Hemolysis Complete, per cent of NaCl	0.27	
Saponin		
Hemolysis Beginning, micrograms of saponin	10	
Hemolysis Complete, micrograms of saponin	43	

* Calculation of the number of cells per gram of cells.
There are 4.76×10^6 cells per c. mm. or 4.76×10^9 cells per milliliter.
Forty per cent of the whole blood consists of cells.
1.0886 is the specific gravity of the cells $\therefore 40 \times 1.0886 = 43.5$ gm. of cells per 100 ml. of blood.
$\frac{4.76 \times 10^9}{0.435} = 10.9 \times 10^9$ or 1.09×10^{10} cells per gram of cells.

Since the physicochemical properties and the structure of the erythrocyte are so closely related in health and in certain diseases, it seems worth while to record as a part of this study of nutrition and chemical growth of children the following hematological observations compiled from values obtained from studies on 19 to 28 healthy children, age 5 to 12 years (Table 106) and discuss them in relation to results obtained in certain diseases. In childhood the determined specific gravity of the normal whole blood is 1.0506, plasma is 1.0272, and erythrocytes 1.0886; the average values of calcium is 10.4 mg. per 100 ml. of blood serum and phosphorus 5.6 mg. per 100 ml. of plasma.

From the measurements of the cell diameters, volume and hemoglobin content it is possible to obtain valuable information on the type of red blood cell formation. In the hypochromic microcytic anemias which are essentially iron deficiency diseases, the erythrocytes on the average are smaller than normal and deficient in hemoglobin. The hyperchromic macrocytic anemias which are due to a deficiency of an essential substance stored in the liver and necessary for the continuance of blood formation in the bone marrow, possess erythrocytes larger than normal and saturated with hemoglobin. It is believed that different factors are involved in the cell stroma formation and the synthesis of hemoglobin.

Osmotic Relations

The blood electrolytes, sodium, potassium and chloride are important in respiratory exchange and in the maintenance of osmotic equilibrium[132,264] within the body. Acidification by increase of carbon dioxide tension and decrease in oxygenation of hemoglobin causes both water and chloride to pass into the cell, whereas alkalinization has the opposite effect. The abnormal types of erythrocytes in hemolytic anemias in childhood[81] suggest that the ionic equilibrium may become upset and that either respiration or osmotic equilibrium or both may become impaired.

The results of the sodium, potassium and chloride determinations in the serum and erythrocytes of 18 healthy children and the concentration of each mineral in the individual corpuscle are given in Table 107. The concentration of the sodium, potassium and chloride was observed to be 142±5, 4±1, 104±6 meq. per liter of serum and 16±6, 115±10 and 52±13 meq. per liter of erythrocytes, respectively.

The mineral composition of the serum is more constant among the individual children of this study than is found in the erythrocytes as shown by the standard deviation values. In the distribution of these minerals between the plasma and red blood cells most of the sodium of the blood resides in the serum and the potassium in the cells while about twice as much chloride is allocated to the serum as to the cells. After computing the cell weight from the erythrocyte volume and specific gravity, computations of corpuscular concentrations may then be made from determining the amount per 100 gm. of cells and the number of cells per unit weight, the latter value being calculated from the cell weight. Inasmuch as wide variations in size and weight of erythrocytes may occur, values expressed in terms of concentrations per unit weight may mask important changes in the individual cell, which may have metabolic or structural significance. It has seemed worth while to show the calculated concentration of sodium, potassium and chlorine in the individual corpuscle for the blood of normal children (Table 108).

TABLE 107

DISTRIBUTION OF SODIUM, POTASSIUM AND CHLORIDE OF BLOOD IN CHILDHOOD

Average of 18 studies

Electrolyte	Serum	Erythrocytes	Corpuscle*
	meq. per liter	meq. per liter	$\times 10^{-13}$ meq.†
Sodium	142	16	14
Potassium	4	115	97
Chloride	104	52	44

* Represents the concentration in an average single red blood cell.

† To calculate meq. per average single corpuscle.
115 meq. of potassium per liter of erythrocytes.
1.0886 is the specific gravity of the cells.

$\frac{115}{1.0886}$ =106 meq. of potassium per kilogram or 0.106 meq. per gram of erythrocytes.

From footnote to Table 106 there are 1.09×10^{10} cells per gram of cells.

$\frac{0.106}{1.09 \times 10^{10}} = 0.097 \times 10^{-10}$ or 97×10^{-13} meq. of potassium per average single red blood cell.

A study of the mineral distribution in the erythrocytes and plasma in children indicates the magnitude of variability among individuals within the age range of 5 to 12 years. It might be used, also, as a measure of the ability of the organism to maintain normal function and equilibrium in the face of changes stimulated by growth and developmental processes and by environmental factors such as food and living conditions.

The sum of the total concentration of anions and cations in the erythrocytes has been calculated for children. The base combined with hemoglobin, on the basis of 44 meq. per liter of cells[264] and the values of Maizels for bicarbonate in serum and cells have been used to calculate the additional ion constituents. The data are expressed both in milliequivalents per liter of cells and per single cell and presented in Table 108. The average excess of positive ions is approximately 15 meq. per liter of cells and 13×10^{-13} meq. per cell. Maizels[224] has shown in his paper on the ion content of blood in anemias that the amount of base present in the red blood cell is greater than that required to combine with the chloride, bicarbonate and hemoglobin of the cell, which suggests that it is in combination with some unknown anion (X^-) present in the cell. A definite parallelism seems to exist between the excess corpuscular base and the undetermined weight of the cell, which is calculated by subtracting the sum of the corpuscular hemoglobin, water, total ions, and total lipids from the corpuscular weight. According to this estimate, approximately three micromicrograms (3×10^{-12} gm.) remained undetermined in the normal red blood cell of children but it may rise to eight in the cells of anemia. This undetermined weight is thought to be composed chiefly of stroma protein in addition to other substances such as non-protein compounds, phosphoric esters and in the case of nucleated cell, of nuclear material.

TABLE 108

ELECTROLYTE CONTENT OF ERYTHROCYTES IN CHILDHOOD

Total cations, meq. per liter of cells	132
Sodium	17
Potassium	115
Total anions, meq. per liter of cells	117
Chloride	53
Bicarbonate*	15
Hemoglobin†	49
Cations minus anions, meq. per liter of cells	15
Excess cations per cell, $\times 10^{-13}$ meq.	13
Undetermined cell weight,‡ ($\times 10^{-12}$ gm.) or micromicrogram	3

* The milliequivalents of bicarbonate were taken as the average values found by Maizels.[224]

† The base combined with hemoglobin calculated on the basis of 44 milliequivalents of base bound by hemoglobin per liter of cells in normal blood.[264]

‡ Calculated by subtracting the sum of the corpuscular hemoglobin, water, total ions, and total lipids from the corpuscular weight.

It has been pointed out that there is a greater range of difference in the individual determinations of minerals contained in the erythrocytes than in the serum. We have sought a possible explanation for this difference. In two groups of children studied, Group 1 was composed of seven children who had been maintained on a fixed dietary and metabolic regimen for eight months preceding the taking of the blood samples, and Group 2 included 11 children who were living in the same environment except they were on the unrestricted diet of the home. Table 109 shows the differences occurring in the mineral content of the serum and red blood cells of the two groups of children.

TABLE 109

THE MINERAL CONTENT OF SERUM AND ERYTHROCYTES OF TWO GROUPS OF CHILDREN ON DIFFERENT DIETARY REGIMEN

Electrolyte	Group of children	Serum		Erythrocytes		Corpuscle	
		Mean	Range	Mean	Range	Mean	Range
		meq. per l.		meq. per l.		$\times 10^{-13}$ meq.	
Sodium	1*	137	130–143	18	11–27	16	10–24
	2†	144	139–147	15	4–23	12	4–20
Potassium	1	4	4–4	122	112–128	109	100–122
	2	4	4–5	111	100–142	91	75–124
Chloride	1	103	97–110	54	43–66	48	42–57
	2	104	96–111	51	35–82	42	28–72

* In Group 1 the children received a fixed dietary and were on metabolic regimen eight months preceeding the taking of the blood samples.

† In Group 2 the children lived within the same institution but ate the regular home diet.

The mean concentration of potassium and chloride is the same in the serum of the two groups of children while the sodium occurred in a slightly higher concentration in the serum of the children on the unrestricted diet (Group 2). When considered in terms of composite weight per liter, the erythrocytes were more concentrated in all three electrolytes in the children on the fixed dietary. Furthermore, there was less variability in the individual determinations of the different mineral constituents in the red blood cells indicating greater standardization of the children included in Group 1 (Table 109). The advantages of determining the corpuscular concentration have been pointed out previously. Applying this method of calculation, the corpuscular weight composition verified the results obtained on the unit weight composition that the erythrocytes of the children on the fixed dietary were not only more concentrated in sodium, potassium and chloride but the range of variations in results is much less.

In order to study the osmotic relationships between erythrocytes and plasma, the average results have been calculated in terms of milliequivalents per liter of serum and cell water (Table 110).

TABLE 110

MEAN OSMOTIC RELATIONSHIPS* BETWEEN ERYTHROCYTES AND PLASMA

Electrolyte	Group of children†	Serum water	Cell water	Corpuscular water
		meq. per l.	meq. per l.	$\times 10^{-13}$ meq.
Sodium	1	146	25	22
	2	153	21	18
Potassium	1	4	169	152
	2	4	154	126
Chloride	1	108	75	67
	2	111	72	59

* Calculations are based on the average serum water of 94.4 per cent given by Peters and Van Slyke[262] and cell water of 66 per cent by weight or 72 per cent by volume as determined in these studies (See Table 106).

† See footnotes, Table 109.

Considering the children in both groups the average concentration of sodium, potassium and chloride in cell water is higher for those who were on a standardized regimen but the greatest difference was found in the potassium values of 169 and 154 meq. per liter of cell water for Groups 1 and 2 respectively. These calculated figures are in agreement with the analytic values previously presented. The results show that the potassium concentration content of the cells is higher for the children who were maintained on the metabolic experiment for eight months (152×10^{-13} meq. as contrasted with 126×10^{-13} meq.). The narrower range of variability and the higher electrolyte content, especially of the potassium and chloride contents of the average individual erythrocyte of the children, suggest that these constituents of the cell may be affected by diet.[43] The sum of the total concentrations of anions and cations in both erythrocytes and plasma of the two groups of children have been calculated. An excess of approximately 21 milliequivalents per liter of water was found in the serum over that in the cells for Group 2. The opposite was true in the highly controlled group where the red blood cells contained approximately 11 milliequivalents in excess of that in the serum.

On the basis of the observations recorded herein the concentration of minerals in the serum of children is strikingly constant, whereas the red blood cells exhibit more variability and appear to be more susceptible of environmental influence such as diet. Inasmuch as the serum appears to maintain a constant state in the mobilization of base it seems that more attention should be given to the composition of erythrocytes in seeking indicators of stages in physiological development, growth and state of health.

Lipids

There are numerous studies on plasma lipids, but few deal with the complete lipid distribution in children. Most of the studies include only one of the lipid constituents such as the extensive studies of Sperry,[324,325] on the free and ester cholesterol relationships. Besides our own reports[17,80,81,384] there have been few observations presented on the lipids in the red blood cells of children. Table 111 presents the blood lipid observations made in this laboratory on the plasma and erythrocytes of children. The average total lipid concentration on a unit basis is essentially similar in the plasma (454 ± 73 mg. per 100 ml.) and the red blood cell (424 ± 30 mg. per 100 gm.) but the percentage distribution of the total lipids is different. Phospholipid and free

TABLE 111

BLOOD LIPID OBSERVATIONS IN CHILDHOOD

Average of 16 studies

Lipid	Plasma	Erythrocyte	Corpuscles*	DISTRIBUTION OF LIPIDS IN BLOOD OF CHILDREN		
				Plasma	Erythrocytes	Stroma†
	mg. per 100 ml.	mg. per 100 gm.	$\times 10^{-12}$ mg.	per cent	per cent	per cent
Total	454	424	389	—	—	—
Phospholipid	136	244	224	30	58‡	65
Neutral Fat	100	51	47	22	12	11
Total Cholesterol	143	116	106	—	—	—
Free Cholesterol	35	97	89	8	23	20
Cholesterol Esters	183	32	29	40	7	4

* Represents the concentration in a single, average red blood cell. Calculations of corpuscle value: There are 424 mg. of lipid per 100 grams of cells or 4.24 mg. per gram of cells.

From footnote to Table 106 we have 1.09×10^{10} cells per gram of cells $\therefore \frac{4.24}{1.09 \times 10^{10}} = 3.89 \times 10^{-10}$ or 389×10^{-12} mg. of lipid per average single red blood cell.

† From blood of adult man.

‡ Cephalin expressed as per cent of phospholipid is 59 per cent.

cholesterol have the highest concentration in the cell, whereas phospholipid and cholesterol ester are highest in plasma. Cholesterol esters occur in low concentrations in the cells and at times may be absent. Free cholesterol has the lowest concentration of all the lipids in the plasma but two to three times this amount may be present in the cells. The neutral fat is a calculated value and varies widely, as shown by standard deviation values. The cells contain about half the amount of neutral fat found in the plasma. The values for the lipid composition of the average individual corpuscle are presented since these may have significance in determining the normality of the intact blood cells.

The actual amount and percentage distribution of the various fractions in the total lipid of the blood plasma and erythrocyte in our studies in childhood is given in Table 111. Although the absolute concentration of the different lipids may vary normally over a considerable range, especially in the plasma, the individual constituents maintain a characteristically constant percentage distribution. In the plasma over two-thirds of the total lipid is made up of cholesterol esters and phospholipids, one-fifth by neutral fat, and less than 10 per cent by free cholesterol. An average of 24 per cent (range 18 to 39 per cent) of the total cholesterol was in the free state. Reports in the literature[253,259,324,325] indicate that maturity of the organism may be a factor in determining the total cholesterol level (it being lower for younger children), and the distribution of the free and combined cholesterol (the cholesterol esters being lower and more variable in infancy) of the blood plasma. The percentage of free in total cholesterol appears to be physiologically constant in normal blood serum or plasma after infancy.

In the erythrocytes, approximately 80 per cent of the total lipid is phospholipid and free cholesterol while the remaining 20 per cent is divided between neutral fat and cholesterol esters in the ratio of 2:1. In the cells, in which the major portion of the cholesterol is in the free state, the average values of free in total for childhood range from 68 to 100 with an average of 84 per cent. The lipid composition of the red blood cells of normal children is not only relatively constant in amount and distribution, but similar in composition to the erythrocyte in adults.

The separation of the phospholipid into its component fractions (lecithin, cephalin and sphingomyelin) in the blood of children has indicated that the division of the phospholipids may prove to be as significant, physiologically, as the distribution of total lipids and cholesterol. More recent methods have enabled the determination of the distribution of the individual phospholipids which will be discussed later.

A study of the lipid content and distribution in the intact erythrocyte and the posthemolytic residue* has furnished important information on cellular structure, especially as it concerns the framework of the cell which appears to be mainly a complex of lipid and protein. The intact cell demonstrates differences in lipid content which do not appear to be related to the amount in the corresponding stroma preparation. Characteristic size and lipid content of the cell is related but the order of magnitude of erythrocyte lipid content is reversed for that of the corresponding stroma preparation. In spite of the differences in absolute values, the type of lipid mixture is essentially the same for all normal stroma preparations. The total lipid of both stroma and erythrocytes is divided as approximately 62 per cent total phospholipid, 11 per cent neutral fat, 22 per cent free cholesterol, and 5 per cent cholesterol ester (Table 111). The importance of the phospholipids in growth, nutrition,[386a] metabolism[241,316] and cell activity has been emphasized, but little is known of the biochemical role of the individual phospholipids, lecithin, cephalin and sphingomyelin. The actual quantities of the total phospholipid and its component fractions, lecithin, cephalin and sphingomyelin have been determined in human blood. The percentage distribution of each of the constituents in terms of total phospholipids also is given in Table 112 for plasma, erythrocytes and posthemolytic residue. Characteristic patterns for the phospholipid partition among lecithin, spingomyelin and cephalin appear to exist for normal plasma, erythrocytes and stroma. In disease (pernicious anemia and lipemia) certain anomalies may occur.[385] Results indicate that cephalin and sphingomyelin may serve as structural phospholipids, whereas lecithin may function metabolically. Further investigation concerning the physiological roles of the individual phospholipids, lecithin, cephalin, and sphingomyelin is needed in health and disease. With the newer analytical technique available, information and reliable data may now be procured.

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* Posthemolytic residues were prepared with comparatively little hemoglobin contamination and in sufficient quantities for detailed lipid and protein analyses. This accomplishment has enabled us to include in our investigation of cellular structure and function, correlated hematologic, physical, and chemical studies of the blood and stroma. In order to interrelate the hematologic, physical and chemical characteristics of the blood with that of the stroma or fixed framework of the erythrocyte, a relatively large quantity of blood, from the standpoint of the human subject, especially children, must be obtained, despite the use of microchemical methods of analysis. From 30 to 50 ml. of blood are required for all the hematologic, physical and chemical measurements. An additional quantity of 250 to 500 ml. of blood must be secured for the stroma preparation. From 5 to 10 gm. of erythrocyte posthemolytic residue are required for lipid and amino acid analyses.

Parpart recently has published a method which, according to the author, yields a stroma preparation of lower iron content. Iron content may not be the only criterion for purity.[260a]

TABLE 112

PARTITION OF TOTAL PHOSPHOLIPIDS IN ADULT HUMAN BLOOD

Phospholipids	Plasma*	Erythrocytes*	Stroma dry weight†	DISTRIBUTION OF TOTAL PHOSPHOLIPID		
				Plasma	Erythrocytes	Stroma dry weight
	mg. per 100 ml.	mg. per 100 gm.	mg. per 100 mg.	per cent	per cent	per cent
Total phospholipids	189	317	10	—	—	—
Total choline phospholipid	134	127	5	71	40	50
Lecithin	99	77	2	52	24	20
Cephalin	55	190	5	29	60	50
Sphingomyelin	35	50	3	19	16	30

* Average of four samples.
† Average of three samples.

Consideration has been given to the relation between cerebroside and neutral fat contents of blood plasma, erythrocytes and stroma.[86] Cerebrosides are known to exist in the blood in small amounts but the methods for determining the lipid partition have not until recently taken the cerebroside fraction into account. It will be recalled that all the lipid unaccounted for as phospholipid or cholesterol and its esters has been included in the calculated neutral fat fraction. By determining the cerebroside in the lipid extracts of the blood plasma, erythrocytes and stroma it is shown that cerebroside occurs in appreciable quantities in the intact cell and posthemolytic residue, but in only small or negligible quantities in the plasma.

The cerebroside content of normal erythrocytes averaged 47 mg. per 100 gm. of cells, thus comprising 9 per cent of the total lipid, whereas in the plasma there were 15 mg. per 100 ml. or 2 per cent of the total lipid. Cerebroside appears to be a structural component of the erythrocyte, for measurable quantities are present in the stroma (10 per cent of the total lipid). In diseases such as pernicious anemia and diabetic lipemia the cerebroside content of the erythrocyte may comprise as much as 20 to 50 per cent of the total lipid.

The lipid and mineral content of tissues have been associated with physiological activity and function. Bloor and his students[21,22,23] have demonstrated that increased physiological activity in a tissue or organ is associated with increased amounts of phospholipid and free cholesterol. Lowered physiological activity, degeneration and retrogression are accompanied by decreased quantities of phospholipids and free cholesterol and increased amounts of neutral fat and cholesterol esters.[382] Similarly Gêrard[111] has suggested that higher potassium to sodium ratios in tissues are related to a higher state of activity. For example, we have found that the lipid and mineral composition of the erythrocytes in the hereditary anemias of childhood differ from the normal. The abnormal erythrocytes such as are found in erythroblastic anemia possess a relatively larger amount of neutral fat, signifying a less active cell. Sickle-cell anemia cells have a relatively increased amount of cholesterol ester suggesting degeneration and retrogression; hemolytic icterus erythrocytes have less phospholipid and more cholesterol esters than normal, indicating signs of diminished activity and degeneration. Confirmatory evidence that physiological activity of the erythrocyte is diminished in these hemolytic anemias may be found in the lower potassium to sodium ratio than normal. This ratio is 2.6 in erythroblastic anemia, 5.9 in sickle cell anemia and 6.5 in hemolytic icterus anemia in comparison with 7.0 in the cells of normal children. These results emphasize the fact that the erythrocyte is an organized entity that experiences reactions such as lowered physiological activity, retrogression and degeneration similar to tissue cells. In view of the results obtained on the blood serum and intact blood cell we have stated:[80] "A consideration of the growth processes (changing structure and function) in the light of both minerals and lipids of the plasma, together with the hematological observations, offers evidence suggesting that the concentration of minerals may indicate the ability of the organism to maintain equilibrium in spite of changing structure and function, whereas the lipids may indicate the progressive stages of development between infancy and maturity. Certain characteristic differences of the plasma lipid found between the neonatal period, preadolescence (five to nine years), and maturity suggest a fruitful field of investigation in extending the present data and exploring other periods of advancement, such as infancy, early childhood, and puberty. On the other hand, the greater constancy of lipids in the erythrocytes among individuals, as well as between children and adults, indicate that they are a more fundamental part of the structure itself and thus not as likely to be influenced by changes accompanying growth and environmental conditions. The minerals of the erythrocytes are probably more mobile constituents and may reflect the nutritional status and certain environmental influences, such as diet."

The average composition of normal adult human posthemolytic residue was found to be 70 per cent protein, 14 per cent lipid, and approximately 5 per cent ash. Nitrogen, lipid and ash analyses indicated that about 90 per cent of the posthemolytic residue could be accounted for as ash and a lipid-protein complex. It was very difficult to wash out the hemoglobin (organic iron) of human stroma in contrast to the posthemolytic residues from erythrocytes of other animals. The presence of organic iron in the dried residues of our series was considered *prima facie* evidence for the presence of hemoglobin and was used as one of the criteria of purity.[260a] The total iron analyses[157] were made on the ashed sample and the inorganic iron determined by the method of Kohler, Elvehjem and Hart.[184] The organic iron content, approximately 0.03 per cent, was obtained by subtracting the inorganic from the total iron present. Calculating the organic iron in the stroma as hemoglobin, assuming that this procedure for calculating iron contaminants is correct, the human posthemolytic residue would appear to contain 5 to 10 per cent hemoglobin contamination. It is believed that at least a portion of the iron may be a component of the true posthemolytic residue[16] and not hemoglobin as a contaminant.

Considering that the stroma is a protein-lipid complex, then human adult stroma has a protein to lipid ratio of five. This high ratio is consistent with the fact that normal human erythrocytes are more resistant to hypotonic hemolytic changes than of certain other types of normal animal cells or of certain blood dyscrasia. Marked alterations in erythrocyte composition do take place in the blood in hemolytic and pernicious anemias. Chemical studies of posthemolytic residues prepared from blood of dogs subjected to a controlled experimental disulfide anemia has proved that abnormalities in the chemical structure of the red blood cells may be induced by anemia.[386] For example, the blood stroma in disulfide anemia showed a content of 71 per cent protein and 12 per cent lipid, giving a protein to lipid ratio of 5.9. In normal dog blood stroma the protein amounted to 57 per cent and the lipid 17 per cent, yielding a protein to lipid ratio of 3.4.

In contrast to the anemias, similar studies on the blood from human polycythemia[85] have demonstrated abnormalities in blood cell structure tending in the opposite direction to that of the anemia cell when compared to the structural makeup of the normal erythrocyte. The protein and lipid contents of the stroma from polycythemia blood ranged from 63 to 67 and 18 to 22 per cent respectively. (The normal stroma contained 70 per cent protein and 14 per cent lipid.) The protein-lipid ratios ranged from 3.0 to 3.7 as compared to 5 for the normal blood stroma. Embryonic blood which has been considered to be similar to that of polycythemic blood from the standpoint of the hematological and physicochemical characteristics of the erythrocyte, yields stroma (beef embryo blood)[85] which contains 69 per cent protein and 20 per cent lipid with a protein:lipid ratio of 3.4. Adult beef posthemolytic residue contains 57 per cent protein and 26 per cent lipid; the resulting protein:lipid ratio being 2.2.

Human blood stromal protein has been analyzed for total nitrogen, total sulfur and seven amino acids including histidine, arginine, lysine, tryosine, tryptophane, cystine and methionine.[7] The average values are presented in Table 113, together with the distribution of the total nitrogen of human blood posthemolytic residue in terms of the nitrogen of the seven constituent amino acids, calculated on the basis of total nitrogen determined on the preparation. The results show that the amino acids determined account for but 27 per cent of the total nitrogen of the protein. Together, the cystine and methionine account for 90 per cent of the total sulfur.

The amino acid analyses of the stroma of bloods from different normal animals show that the general pattern of the amino acid composition is similar for all mammals. Significant, but dissimilar, deviations from normal may occur in the amino acid composition of the posthemolytic residue protein from polycythemia vera and cardiac polycythemia and beef embryo.[85] The differences in the protein and lipid composition of the stroma of embryo bloods from that of the normal adult has stimulated a study which we now have in progress; the pattern of the chemical composition of tissues during growth. The fairly low total basic amino acid content of stroma protein serves to demonstrate its dissimilarity to hemoglobin and is not inconsistent with the belief that the stroma may contain significantly large amounts of acidic amino acids with which the excess cell cation[7,168,386] is combined.

TABLE 113

NITROGEN, SULFUR, AND AMINO ACID COMPOSITION OF LIPID-EXTRACTED STROMA FROM ADULT HUMAN BLOOD		NITROGEN OF AMINO ACIDS IN STROMA	
	Per cent of stroma		*Per cent of total stroma nitrogen*
Total nitrogen	13	Nitrogen accounted for	27.0
Total sulfur	0.7		
Histidine	2.1	Histidine	4.3
Arginine	4.8	Arginine	11.8
Lysine	3.8	Lysine	5.8
Tyrosine	2.7	Tyrosine	1.7
Tryptophane	1.2	Tryptophane	1.2
Cystine	0.9	Cystine	0.8
Methionine	1.8	Methionine	1.4

Blood Globin

Chemical analyses of the red blood cell shows that 90 to 95 per cent of the total solids consists of hemoglobin and therefore is of great importance in determining the composition of the whole erythrocyte. Regardless of whether the hemoglobin is held free in the erythrocyte within an enclosing cellular membrane or absorbed in a sponge-like framework, it may be released in whole or in part upon hemolysis. The heme groups are identical among hemoglobins hence differences in hemoglobins must find their basis largely in differences in the globin portion of the protein moiety.[8,20,358]

The sulfur containing amino acid compositions of hemoglobins are known to vary in different species. Table 114 shows the results of the total nitrogen, total sulfur and cystine and methionine analyses of adult human blood globin. Cystine and methionine determined from results of the amino acid analyses account for 97 per cent of the total sulfur in human globin.

TABLE 114

COMPOSITION OF HUMAN BLOOD GLOBINS

Values in per cent

Nitrogen	16.58	Methionine	1.23
Total sulfur	0.60	Cystine-sulfur	0.33
Cystine	1.21	Methionine-sulfur	0.26
Total sulfur determined	97		

Platelets

The platelets make up one of the important groups of formed elements in the blood. By virtue of certain physical and chemical characteristics they play an important role in blood clotting[153] but the *modus operandi* of platelets in blood coagulation is not well defined. When bleeding occurs the platelets aid physically in fibrin formation and clot retraction[94, 153] and may liberate specific clot-aiding substances during active participation in the coagulating process. When blood is shed the platelets show swelling, agglutination, excrescence formation, and partial disruption.[94,347] Their physicochemical characteristics under these circumstances resemble the osmotic phenomena of phospholipids.[192] Although a thermostable thromboplastic agent which has been identified with cephalin[47,71,95] has been observed during platelet disintegration, the determination of the exact nature of the clot-aiding substances must await future investigations.

The majority of studies of the role played by the platelets in the mechanism of blood clotting have been concerned primarily with platelet origin, morphology and physiology.[194] Because of our particular interest in cellular structure as it pertains to growth and nutrition and the genealogical relation of platelets to other formed elements of the blood we have approached the study of platelets through the determination of their chemical composition. Special emphasis has been placed upon the lipid and nitrogen content and the lipid partition since previous investigations indicate the importance of these substances, not only in cellular structure but in blood clotting.[83]

The chemical studies of the platelets of children have included total lipid, phospholipid, free cholesterol, cholesterol esters, neutral fat and protein (Table 115). The pooled sample of platelets was obtained from a group of nine children who were under controlled conditions of food intake and environment during studies of nutrition and chemical growth. Values based upon per cent of dry weight of platelets are: protein, 56; total lipid, 13; phospholipid, 9, or over two-thirds of the total lipid; free cholesterol, 3, or about one-fourth of the total lipid; cholesterol ester, 0; neutral fat, 1.

TABLE 115

COMPOSITION OF NORMAL PLATELETS IN CHILDHOOD*

Constituent	Per cent of dry weight	Per cent distribution of total lipid
Protein	56	—
Total lipid	13	—
Phospholipid	9	69
Free cholesterol	3	23
Cholesterol ester	0	0
Neutral fat	1	8

* A pooled platelet sample from nine children, ages four to eight years.

From the viewpoint of cellular structure and blood cell interrelationship, the average composition of adult human platelets is presented with the composition of other formed elements in the blood, namely leucocytes, erythrocytes and red cell stroma, in Table 116. The analyses of the leucocytes have been taken

TABLE 116

COMPOSITION OF FORMED ELEMENTS IN ADULT HUMAN BLOOD

	Platelets	White cells*	Red cells	Red cell stroma	DISTRIBUTION OF TOTAL LIPID			
					Platelets	White cells†	Red cells	Red cell stroma
	per cent of dry weight				per cent			
Protein	64	69	90	70	—	—	—	—
Total lipid	15	22	1.3	14.1	—	—	—	—
Phospholipid	11	—	0.7	9.6	73	60	54	68
Free cholesterol	2	—	0.3	2.3	13	16	23	16
Cholesterol esters	1	—	0.1	0.5	7	10	8	4
Neutral fat	1	—	0.2	1.7	7	14	15	12
Cephalin‡	—	—	—	—	68	—	58	48

* From Haurowitz and Sladek.[127]

† From Boyd.[31]

‡ Per cent of total phospholipid.

from the scanty data in the literature[31, 127] and the remainder of the determinations are from our own studies.[16, 80, 82, 269] On the basis of per cent of dry weight, the platelets and erythrocyte stroma contain similar amounts of lipids, whereas the erythrocytes possess only about one-tenth as much lipid. In spite of the characteristic differences in the absolute lipid values, the percentage distribution of the total lipid in the platelets, leucocytes, erythrocytes and posthemolytic residue, is consistent for all. It has been suggested that the uniformity of the lipid patterns may indicate a characteristic chemical structure and close biological interrelationship of the formed elements in the blood. Surely the problem is one which is worthy of further investigation.

Iron

The red blood cell and hemoglobin, being very labile constituents of the blood, may reflect nutritional defects readily, especially in respect to iron and dietary vitamin deficiencies and to disturbances of gastrointestinal and infectious origin. We have included iron balances (see p. 204) as a means of measuring the sufficiency of iron consumption and of determining the degree of iron utilization by the body. Because copper, and possibly manganese, zinc and nickel may participate in either hemoglobin formation or in the catalytic enzymatic processes in connection with the blood's nutritive activities in the organism, copper, manganese, nickel and zinc balances also have been determined (see p. 198).

Iron analyses of body tissues, the studies of hemoglobin formation and of the heme porphyrin pigment of individual cells, demonstrate the widespread distribution of iron in the organism and the important role it plays in the physiological processes of life. Two known forms of iron exist in the blood, one fraction in the form of the firmly fixed iron in the hemoglobin molecule and the other non-hemoglobin fraction in a loosely combined form. Little is known of the origin and function of the free iron fraction in the blood although the following speculations have been offered: that it is an intermediate form between hemoglobin in the circulation and the iron in the liver, spleen and bone marrow, that it serves as transport iron, that it functions as a respiratory pigment and carries oxygen in the blood cell, and that it acts as a catalyst, aiding in blood regeneration. Whatever the function may be of the loosely combined iron of the red blood cell, the two fractions of iron appear to have a common source of replenishment. Although the non-hemoglobin iron does not originate from the hemoglobin, and is independent of the hemoglobin content of the blood, yet iron and other specific mineral salts stimulate the hematopoietic organs in some specific way quite apart from their possible function as structural units in the formation of the red blood cells. In this connection it should be pointed out that iron also appears to be a true component of the red blood cell stroma.[7,16]

Hemoglobin is a compound of protein (globulin) and iron porphyrin pigment. The pigments formed by the combination of iron with various porphyrins are called hemes and a group of them are closely related to hemoglobin. Although the heme pigments have in common similar iron porphyrin nuclei they all have individual peculiarities due in part to the specific natures of the substances to which they are united. In addition to hemoglobin another heme pigment has been discovered in quantities of only one part in a hundred million in cells. It too acts as a respiratory catalyst. In physiology the chemical properties of the heme pigments are of interest in the understanding of the respiratory function of the blood and more recently in connection with cellular respiration.

From the standpoint of porphyrin chemistry, Hans Fischer and his students[97] have contributed extensive investigations on the organic and physical characteristics of porphyrins especially pertaining to hemoglobin and chlorophyll. The physiologist and biochemist are concerned with the intact heme molecule and how it may be affected by the nitrogenous compound to which it is attached. It has been shown that hemoglobins of different animals differ in the composition of the globin portion of the molecule.[8,20]

The remarkable powers of hemoglobin are associated with it as a carrier of oxygen and as a buffer. Heme itself cannot combine with oxygen loosely but when coupled as hematin with globin, it acquires the power of uniting with and dissociating itself from oxygen according to the needs of the organism. Because hemoglobin is not a catalyst of oxidation but a specific carrier of oxygen and CO_2, the relative quantity contained in the blood cell is an important factor in health and disease.

Hematochemical Studies in Nutritional Anemia

It has been pointed out that by measurements of the red blood cell diameter, cell volume and hemoglobin content it is possible to obtain much valuable information on the normality or type and degree of abnormality of the red blood cell and of hemoglobin formation (see p. 28). Moreover, it has been shown that a study of the physicochemical properties of the erythrocyte together with chemical determinations upon the intact cell and upon the plasma give specific information upon the independency of the different factors which control hemoglobin synthesis and cell stroma formation. The most commonly observed form of blood condition which reflects the adequacy of iron intake and iron utilization is nutritional anemia which is commonly referred

TABLE 117

COMPARISON OF HEMATOLOGIC OBSERVATIONS ON NORMAL AND NUTRITIONAL ANEMIA BLOOD IN CHILDHOOD

Observation	Normal	Nutritional anemia
Erythrocytes		
Whole blood, millions per c.mm.	4.76	4.5
Per gm. cell $\times 10^{10}$	1.09	1.51
Hemoglobin		
Whole blood, gm. per 100 ml.	13	6
Single cell, micromicrogram	28	14
Index*	0.325	0.222
Corpuscular Measurements		
Hematocrit, per cent	40	27
Volume, $c\mu$	84	60
Weight, micromicrogram	91	65
Diameter, μ	7.2	7.7
Thickness, μ	2.1	1.2
Diameter to thickness ratio	3.4	6.8
Volume to thickness index†	1.15	0.67
Resistance to hypotonic NaCl, per cent		
Hemolysis Beginning, per cent NaCl	0.41	0.45
Hemolysis Complete, per cent NaCl	0.27	0.18
Resistance to saponin, micrograms		
Hemolysis Beginning, μ of saponin	10	9
Hemolysis Complete, μ of saponin	43	9

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* Hemoglobin index obtained by dividing the hemoglobin per cent by the red blood cell hematocrit value.

† To determine volume to thickness index (in terms of Haden's normal adult).
Diameter $=7.2$. From Figure 2, page 312, the corresponding indice is 0.815 $\therefore$ normal cell volume $=0.815 \times 90$ (normal adult volume) $=73$.

For normal child index $=\frac{84}{73}=1.15$.

to as hypochromic microcytic anemia. This condition may exist in various degrees of severity and it is essentially an iron deficiency disease. Contrary to the common views, the defects in the blood are not confined to the plasma and hemoglobin portion of the cell but abnormalities may occur in the structural portion of the erythrocyte also, as is shown by interrelated hematological observations, lipid and mineral analyses, the degree of alteration depending upon the severity of the condition. A study of the dimensions of the cells including volume, diameter and thickness shows that the red blood cell in nutritional anemia in childhood is smaller than normal and more discoidal in shape, the diameter to thickness being 3.4 and 6.8, and the volume to thickness ratio being 1.15 to 0.67, respectively, in health and iron deficient anemia (Table 117). The small cell of hypochromic anemia seems to be less resistant to osmotic hemolysis than its discoidal shape would indicate. The hemoglobin index, obtained by dividing the hemoglobin per cent by the red blood cell hematocrit value, is approximately two-thirds the normal values in the microcytic anemia.

TABLE 118

COMPARISON OF BLOOD LIPIDS IN NORMAL AND NUTRITIONAL ANEMIA BLOOD IN CHILDHOOD

	Plasma		Erythrocytes		Corpuscle	
	Normal	Anemia	Normal	Anemia	Normal	Anemia
	mg. per 100 ml.		mg. per 100 gm.		$\times 10^{-12}$ mg. per cell	
Total lipid	454	611	424	541	389	369
Phospholipid	136	152	244	302	224	207
Neutral fat	100	215	51	39	47	29
Cholesterol, total	143	164	116	179	106	119
Free	35	48	97	148	89	99
Ester	182	196	32	52	29	34

The physical contour of the erythrocyte may be related to chemical composition; contrary to the current view that the erythrocyte remains constant under varying pathological conditions common to man, a study of the lipid composition of the red blood cell in simple nutritional anemia may appear to demonstrate significant variations from normal in some of the lipid constituents (Table 118) on the basis of absolute values. The absolute values on the basis of milligrams per 100 grams of red blood cells show augmented total lipid values in this particular anemia due to increased free cholesterol, cholesterol esters and phospholipid. Inasmuch as a change in the size and the weight of the erythrocyte occurs in nutritional anemia it is important to express the values from the chemical analysis in terms of concentration per individual cell as well as per unit weight. In nutritional anemia there are 1.51×10^{10} erythrocytes in one gram of packed cell whereas normally there occurs 1.09×10^{10} cells. If the values of lipids per 100 gm. of cells are calculated, therefore, on the basis of corpuscular composition, each cell contains approximately the same lipid value as the normal. Although the whole blood shows a reduced hemoglobin of six grams per 100 ml. as compared with 13 grams for the normal child, on the basis of corpuscular concentration the hemoglobin is half the normal value, 14×10^{-12} grams as compared with the normal of 28×10^{-12} grams.

In summary, the individual hypochromic anemia cell is reduced in volume and deficient in hemoglobin but the structural lipid component is there in a normal quota. The cellular composition values demonstrate the importance in determining the chemical results on this basis in addition to those of the absolute concentration. In addition to the absolute concentration of lipids in the erythrocyte, the percentage distribution of the various lipid constituents in the total lipids, which normally maintains a characteristic pattern, is not altered in nutritional anemia.

Characteristic changes occur in the plasma of nutritional anemic children. There is a moderate increase in the neutral fat which accounts for a large part of the increased total lipid value of the blood. Although the absolute plasma lipids may vary normally over a moderate range, the percentage of the lipid fractions on the basis of total fat are relatively constant (Table 119).

TABLE 119

PERCENTAGE DISTRIBUTION OF THE TOTAL LIPID IN NORMAL AND NUTRITIONAL ANEMIA BLOODS IN CHILDHOOD

LIPID FRACTIONS	PLASMA		ERYTHROCYTES	
	Normal blood	Nutritional anemia	Normal blood	Nutritional anemia
Phospholipid	30	25	58	56
Neutral fat	22	35	12	7
Free cholesterol	8	8	23	27
Cholesterol esters	40	32	7	10

In nutritional anemia, however, the percentage distribution of the individual components of the plasma total lipids is markedly altered.

Determinations of sodium, potassium and chloride in the red blood cells and the plasma are of interest, since they are so important in respiratory exchange and maintenance of osmotic equilibrium (Table 120). The range and average value of chloride are within normal limits in both the cells and plasma. However, on the basis of corpuscular concentration the chloride content is lower in simple anemia, 35×10^{-13} milliequivalents per cell in comparison to 44×10^{-13} meq. per cell in the normal. Although absolute values for potassium may be higher in the plasma and cells, on the basis of corpuscular concentration the hypochromic cells are significantly less. Sodium is variable appearing within normal range in both absolute and cellular concentration.

TABLE 120

COMPARISON OF DISTRIBUTION OF SODIUM, POTASSIUM AND CHLORIDE IN NORMAL BLOOD AND NUTRITIONAL ANEMIA BLOOD IN CHILDHOOD

Mineral	Serum		Erythrocyte		Corpuscle	
	Normal	Anemia	Normal	Anemia	Normal	Anemia
	meq. per liter		meq. per liter		$\times 10^{-13}$ meq.	
Sodium	142	133	16	15	14	11
Potassium	4	6	115	129	97	82
Chloride	104	104	52	54	44	35

Calculations of the cation and anion contents of erythrocytes in anemia brings out an interesting relationship between excess corpuscular base and the undetermined weight of the cell (Table 121). The undetermined weight of the cell, which is considered

TABLE 121

A COMPARISON OF TOTAL CATION AND ANION CONTENT OF ERYTHROCYTE IN NORMAL AND NUTRITIONAL ANEMIA BLOODS IN CHILDHOOD

Observations	Normal	Anemia
Total cations, meq. per liter cells	132	144
Total anions, meq. per liter cells	117	103
Cations minus anions, meq. per liter cells	15	41
Excess cations per cell $\times 10^{-13}$ meq.	13	25
Undetermined cell weight $\times 10^{-12}$ gm.	3	8

to be composed primarily of stroma protein, non-protein nitrogen compounds and phosphoric esters, is greatly increased, 8×10^{-12} grams in the anemia as compared 3×10^{-12} gram in normal blood.

Considering the lipid and mineral contents of the erythrocyte in relation to physiological activity and efficiency, the cells of nutritional anemia appear to be normal in structural material (i.e., stroma) but are reduced in size and metabolic efficiency because of the reduced quantity of hemoglobin, not because of retrogression or degeneration of the cell itself. The potassium to sodium ratio in the cell was within the normal range and there was a normal pattern of lipid distribution. From the standpoint of cell structure and function, from the physiological activity of hemoglobin, these studies on the chemistry of the red blood cell have a definite relation to iron consumption and utilization in the body.

CHAPTER X

METHODS

ANALYSIS OF FOOD, URINE AND FECES

ACCURACY in the collection and preservation of the material whose composition is to be determined is prerequisite to obtaining valid data from analyses. In the preparation of the materials for analysis several additional factors must be considered and methods selected which will provide maximum accuracy with the staff and equipment available and under the conditions of the investigation. The purpose of a study will determine the number and kind of analyses to be performed; these, in turn, decide the method of preparation of materials for analysis.

If dried material is necessary for analysis or if it is desirable to preserve samples for later analysis, the most satisfactory desiccation procedure is evaporation of "fresh-frozen" food, feces or urine under high vacuum.* Preparation by this method[101] of all the metabolic balance material for several subjects involves a large outlay for equipment and high cost for preparation, although, in an investigation over a period of months, greater staff efficiency may result by deferring the majority of analyses until collections are completed. We have found desiccation by this method of great value in determining the fat and complex carbohydrate distributions in feces, the energy value of urine, and the "heavy metals" in foods, urine and feces. Other methods for preparation yield analytic material almost equally satisfactory for other determinations.

Preparation of Analytical Material

It is essential that the samples of food for analysis adequately represent the actual food intake of the experimental subjects for definite periods and that urine and feces samples accurately represent the excretion from the food ingested. The amounts of urine and feces available for analysis are limited by the length of the balance periods and by the size and individual characteristics of the subjects.

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* "Cryochem" apparatus manufactured by F. J. Stokes Machine Company, Philadelphia, Pa. The drying is accomplished under vacuum pressure of 150-200 microns. We found it convenient to adapt the equipment to provide for drying eight samples in 250 ml. pyrex, wide-mouth centrifuge bottles.

Food: The diets given to experimental subjects may be analyzed either as composite samples of the total daily food intake or each food may be prepared for separate analysis. The composite does not provide an estimation of the variations in the mineral content of individual foods but does give more accurate values for the daily intake of the various elements by reducing the number of determinations and therefore minimizing the chance of summation of errors and lessening the number of sources of error. Single foods which have characteristics that introduce difficulties in analysis of the composite, or whose bulk is large in the diet, may be analyzed separately and the values added to those for the composite. In the studies of normal children, a duplicate of each diet, exclusive of milk, potato, butter, sugar and salt, constituted the composite. Milk and potato were analyzed separately; then, the values were added to those for the composite. Butter, sugar and salt were not analyzed. The salt used was chemically pure sodium chloride and butter oil was obtained by warming unsalted butter and washing thoroughly with distilled water.

Two identical food composites, each amounting to one day's consumption of basal foods were accumulated during each balance period (p. 50). One of the composites was placed in a large, weighed evaporating dish, an equal volume of alcohol added and the whole dried in a steam oven at 60° C. The alcohol dried material was weighed, ground in a ball mill and 20 gram samples ashed in a muffle furnace at 500° C. for 36 hours. After ashing, the residues were dissolved in dilute hydrochloric acid (1:2) and filtered through ashless filter paper into 100 ml. volumetric flasks, washing five or six times with distilled water. The ash solutions were used for mineral analyses.

The second composite was weighed, finely ground in a food chopper, and thoroughly mixed. (The food chopper used in grinding the foods must be thoroughly washed with distilled water and the washings added to the ground composite before mixing.) The suspension of finely ground mixed food was weighed and 5 to 10 per cent of the wet material (by weight) placed in a porcelain dish and dried in a vacuum oven at 40° C. The dry material was weighed, pulverized in a mortar and stored for

ANALYTICAL MATERIAL FROM FOOD COMPOSITES

Diet:

Date of Preparation:

	COMPOSITE I	COMPOSITE II	
Wet weight of food + dish (gm.)	2004	2051	
Weight of dish (gm.)	1050	1095	
Wet weight (gm.)	954	956	
Wet weight of food + wash water + dish (gm.)		3356	
Weight of dish (gm.)		1122	
Wet weight (gm.)		2234	
		VACUUM DRIED	ACID DIGEST
Wet weight + dish (gm.)		465	1060
Weight of dish (gm.)		223	393
Wet weight (gm.)		242	667
	OVEN DRIED		
Dry weight + dish (gm.)	1197	256	
Weight of dish (gm.)	885	223	
Dry weight of sample (gm.)	312	33	
Average daily dry weight (gm.)	312	305 (33 × 2234 ÷ 242)	

INDIVIDUAL FOODS

(Solid or Liquid)

Food	**Milk**				
Wet weight + container (gm.)	343				
Weight of container (gm.)	239				
Wet weight (gm.)	104				
Dry weight + container (gm.)	55.98				
Weight of container (gm.)	43.41				
Dry weight (gm.)	12.57				

energy determination and fat, carbohydrate, lignin, cellulose, hemicellulose, carbon, sulfur and chlorine analyses.

Twenty-five to 35 per cent* of the wet material from the composite was weighed into a two-liter beaker and digested on a steam bath over night with one-fifth to one-tenth its volume of concentrated sulfuric acid.[327] Under this treatment all of the food either dissolves or breaks into a very fine suspension. The digest was sieved into a volumetric flask, made to volume and saved for subsequent removal of aliquots for the total nitrogen determinations.

In preparing the individual foods for analysis the samples of solid foods were dried *in vacuo*, at 40° C., ground in a mortar, mixed and ashed. For analyses upon liquid foods, which require dried samples, the food was dried in a desiccator or vacuum oven or weighed into 250 ml. centrifuge bottles, frozen with dry ice, and dried from the frozen state.

Urine: The importance of accurate urine collection in metabolic balance studies cannot be overemphasized (p. 51), for inaccuracy in accumulating the 24-hour composites of kidney excreta produces far greater errors than those inherent in the analytical methods. Each day's collection of urine should be transferred from the collecting jar to a one-liter graduate (larger if necessary) and its volume recorded before making to the next 100 ml. to simplify subsequent calculations. One-half of the urine is used for the determinations carried out daily and the other half transferred to a glass-stoppered gallon bottle and kept in the refrigerator. All urine samples should be kept covered with a layer of toluene. (Volumes can be read in a graduate, exclusive of the toluene over the urine.) Urine for the daily determinations may be placed in a quart jar (glass cover) and duplicate portions taken for the determinations of total nitrogen, organic acids, *pH*, and titrable acidity. The remainder is used for the nitrogen partition (ammonia, urea, uric acid, creatine, and creatinine).

After collections for the balance period are complete, the composite urine specimen should be thoroughly mixed and one quart jar filled, sealed with paraffin and placed in reserve in a cool room (18° C.). After measuring the specific gravity of the composite a number of 75 ml. samples which will provide a total of approximately 10 gm. of dried material should be transferred to 250 ml. centrifuge bottles for drying from the frozen state under vacuum. Seventy-five milliliter samples provide the maximum surface area for evaporation in 250 ml. centrifuge bottles with the equipment used in the studies of normal children. The remainder of the composite is used for determination of the various chemical elements.

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* The remaining 55 to 70 per cent of the food composite would provide sufficient material for all the analyses carried out upon the steam-oven dried composite, however, drying the remainder of the diluted composite (the volume is frequently more than doubled by the addition of wash water) introduces more difficulties in procedure than is warranted by the conservation of food represented by obtaining only one composite.

ANALYTICAL MATERIAL FROM URINE

Subject: Balance period:

	Daily Urine Volumes					
	1	2	3	4	5	average
Urine collected (ml.)	1042	860	887	933	993	943
	(Collected volume minus water used to transfer)					
Urine for day (ml.)	892	710	737	783	843	793
Made-up volume (ml.)	1100	1000	1000	1000	1000	1020
Volume of composite for drying from frozen state (ml.)	75					
Weight of dried sample and bottle (gm.)	99.609					
Weight of bottle (gm.)	96.251					
Dry weight of sample (gm.)	3.358					
Dry weight per ml. composite (gm.)	0.0448	(Dry weight of sample ÷ volume of sample)				
Average dry weight per day (gm.)	45.70	(Dry weight per ml. of composite × average composite volume per day)				

Feces: Fecal material was quantitatively transferred from the individual jars in which each stool was collected, to the proper composite collection jar. All fecal material containing red dye was retained for the current balance period. The uncolored feces were pooled with those collected for the preceding period, during the previous five or six days, thus completing the balance period composite. (See p. 37)

Great care must be given to the most precise separation of the colored from the uncolored feces. The most exact chemical procedure carried out by the most highly trained chemist with expensive precision equipment can yield data for metabolic balance interpretation that is both erroneous and misleading if the separation of the feces has not been exact. Experienced subjects often can assist the chemist by manipulating the collection jar in such a way that feces are deposited in a line and not permitted to pile up into a large, indistinguishable mass.

The individual defecations, after weighing in the original collection jar, were transferred with distilled water into a two- or four-quart tared specimen jar. All of the stools for each balance period were added to the composite which was kept, covered, in a refrigerator until complete. The contents of the jar containing the feces for the entire period were mixed with an electric mixer and the weight of the homogeneous mixture of feces and distilled water (used to transfer) recorded before preparing the feces for analysis. A portion of the feces composite was digested with sulfuric acid, over a steam bath, another portion was prepared by evaporating from the frozen state, and the remainder was dried with alcohol in a steam oven (60° C.). The material dried from the frozen state is most satisfactory for the determination of complex carbohydrates and fecal lipid distribution.

ANALYTICAL MATERIAL FROM FECES

Subject:

Carmine marker given: **7:00 A.M. 11-7**

Period:

Carmine marker ejected: **3:30 P.M. 11-7**

Date prepared:

Date	Time	Weight of stool, container and spatula	Weight of container and spatula	Wet weight of feces
11-7-39	**3:30 P.M.**	**617**	**575**	**42**
11-8-39	**6:10 P.M.**	**718**	**594**	**124**

Wet weight of feces for period (gm.)	**2800**
Average fecal wet weight per day (gm.)	**560**
Weight of mixed composite + wash water + jar (gm.)	**3710**
Weight of jar (gm.)	**1581**
Wet weight of mixed composite + wash water (gm.)	**2129**
Mixed composite taken for acid digest + jar (gm.)	**736**
Weight of jar (gm.)	**333**
Weight of acid digest sample (gm.)	**403**
Mixed composite taken for drying from frozen state + jar (gm.)	**360**
Weight of jar (gm.)	**241**
Wet weight of sample to be dried from frozen state (gm.)	**119**
Dry weight of sample dried from frozen state + jar (gm.)	**50.44**
Weight of jar (gm.)	**43.33**
Dry weight of sample dried from frozen state (gm.)	**7.11**
Average daily dry weight from sample dried from frozen state	**25.44** **$(7.11 \times 2129 \div 119 \div 5)$**
Mixed composite remaining for oven drying (gm.)	**1607** **$(2129-403-119)$**
Dry weight of feces over dried + dish (gm.)	**1256**
Weight of dish (gm.)	**1169**
Dry weight of oven dried feces (gm.)	**87**
Average daily dry wt. from oven dried feces	**23.05** **$(87 \times 2129 \div 1607 \div 5)$**

ENERGY

Energy is determined by the oxygen bomb calorimeter method* upon dried samples of food, feces and urine. Corrections must be deducted for the heat of combustion of the fuse wire oxidized in the bomb, and the energy value of the free acid formed in the bomb by oxidation of some of the nitrogen during combustion. The heat produced by oxidation of the fuse wire is determined by the factor 2.8, which represents the calories produced for each centimeter of fuse wire burned. The energy value of the free acid left in the bomb is determined directly by titration with sodium carbonate. According to the usual convention in calorimetry this free acid is all assumed† to be nitric acid and the correction is made for oxidation of nitrogen to N_2O_5 and its heat of hydration to HNO_3. The sum of these corrections is subtracted from the total calories produced in the bomb, to obtain the energy value of the material. For foods and feces the above corrections result in the total energy value of the material, but for urine an additional correction is required, the energy loss as ammonia in drying the material. This loss is determined as the difference between the nitrogen content of fresh and dried samples of urea. It is assumed that this loss of nitrogen represents loss of combustible compounds of the urine and a caloric value is assigned to the nitrogen loss, based on the calorie to nitrogen ratio of urea, that is, 5.447 calories per milligram of nitrogen. The method for determining this loss in caloric value is shown on the accompanying data sheet. The total sulfur and chlorine contents of foods and feces are also determined upon the bomb washings from the energy determination (see p. 269). Total urinary sulfur and chlorine may be determined upon the bomb washings, although the procedure given for fresh urine (p. 270) is preferable.

Procedure

Energy in urine: Dry 75 ml. of urine from the frozen state under vacuum of 100–200 millimicrons pressure (McCleod

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* The method is adapted to biological material from the instruction manual furnished with the Parr Oxygen Bomb Calorimeter, manufactured by the Parr Instrument Co., Moline, Illinois.

† While the results obtained by applying the conventional correction factor are of high accuracy the assumption upon which the correction is based is not rigidly correct. A portion of the free acid in the bomb results from oxidation of organic sulfur, however, some of the acid formed on combustion may be neutralized by an excess of positive minerals in food and excreta.

Gauge). This quantity of urine usually yields about 1.5 gm. of dry solid matter. Weigh 0.3 to 0.5 gm. of the dried urine into an illium capsule; place in the oxygen bomb; close bomb, and inject 25 atmospheres of oxygen. Record the length of the fuse wire. Place the charged bomb in the calorimeter and pour two liters of distilled water into the container. Close calorimeter and start electric water stirrer. Take temperature readings each minute, accurate to 0.1° F., until a constant reading is obtained for three consecutive minutes.* Record the constant reading as the initial temperature. Ignite the charge in the bomb and read

DETERMINATION OF ENERGY REPRESENTED BY NITROGEN LOSS IN DRYING URINE

Analyzed material: **Urine** Subject: **A. B.**

Date of analysis: **10-21** Balance period: **14**

	SAMPLE I*	SAMPLE II	SAMPLE III
Volume of urine dried (ml.)†	**75**		
Weight of dried urine (gm.)	**3.31**		
Volume fresh urine per gm. dry material (ml.)	**22.6586**	(Volume urine dried ÷ weight of dried urine)	
Weight of sample (mg.)	**0.1009**		
Fresh urine represented by sample (ml.)	**2.28625**	(Weight of sample × volume urine per gram dry sample)	
Volume HCl for titration (ml.)	**13.48**		
Normality of HCl	**0.1304**		
Volume of normal HCl equivalent (ml.)	**1.757792**	(Volume HCl × normality of HCl)	
Nitrogen in sample (mg.)	**24.609088**	(Volume of *N* HCl equivalent × 14)	
Nitrogen per ml. urine in sample (mg.)	**10.76**	(Nitrogen in sample ÷ volume of urine represented in sample)	
Nitrogen per ml. fresh urine (mg.)‡	**10.78**		
Loss of nitrogen per ml. from drying (mg.)	**0.02**	(Nitrogen per ml. fresh urine minus nitrogen per ml. urine after drying)	
Energy loss per ml. urine (cal.)	**0.10894**	(Loss of nitrogen per ml. urine × 5.447)	

* Illustrative values were calculated by machine. Some imply greater accuracy than the procedure warrants (See section on Treatment of Data).

† From record of drying.

‡ From record of Kjeldahl nitrogen determination.

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* Temperature of the outside jacket must be brought to, and maintained at the temperature of the bomb inside, by the circulating water.

temperature every 30 seconds until the maximum rise is noted. Record as maximum temperature. (In practice, it is well to record each temperature reading on the reverse side of the energy data sheet and to continue to record temperature readings for several minutes after the apparent maximum rise is observed.

DETERMINATION OF ENERGY

Analyzed material: **Urine**

Average daily volume (ml.): **1040**
Average daily dry wt. (gm.)

Subject:

Balance period:

Date of analysis:

	SAMPLE I	SAMPLE II	SAMPLE III
Volume of fresh urine per gram dry material (ml.)*	**22.6586**		
Dry weight of sample (gm.)	**0.5006**		
Volume of sample (ml.)	**11.3429**	(Dry weight ×vol. of urine per gm. dry material)	
Initial length of fuse wire (cm.)	**10**		
Final length of fuse wire (cm.)	**4.9**		
Fuse wire burned (cm.)	**5.1**		
Acid titration (ml.)	**8.80**		(1 ml. =1 cal.)
Correction for fuse wire burned (cal.)	**14.28**		(2.8 cal. per cm.)
Total correction (cal.)	**23.08**	(Acid titration +correction for fuse wire)	
Initial temperature reading (°F.)	**72.04**		
Maximum temperature reading (°F.)	**72.77**		
Total rise in temperature (°F.)	**0.73**	(Corrected maximum temperature −corrected initial temperature)	
Total rise in temperature (°C.)	**0.40**	(Total rise in temperature in °F. ×0.556)	
Heat evolved (cal.)	**976.40**	(Total temp. rise (°C.) ×water equivalent of instrument)	
Determined energy value of sample (cal.)	**953.32**	(Heat evolved −total correction)	
Energy loss per ml. urine (cal.)*	**0.10894**		
Energy loss in drying sample (cal.)	**1.24**	(Energy loss per ml. ×volume of sample)	
Total energy value of sample (cal.)	**954.56**	(Determined energy value +energy loss in drying urine)	
Energy value per ml. urine (cal.) Energy value per gm. dry wt. feces or food (cal.)	**84.15**	(Final energy value ÷volume of urine or dry weight in sample)	
Energy value per day (cal.)	**87516**	(Energy value per ml. or gm. dry wt. ×daily volume or dry weight)	

Both initial and maximum temperature readings can be transferred to the data sheet after the combustion is completed.)

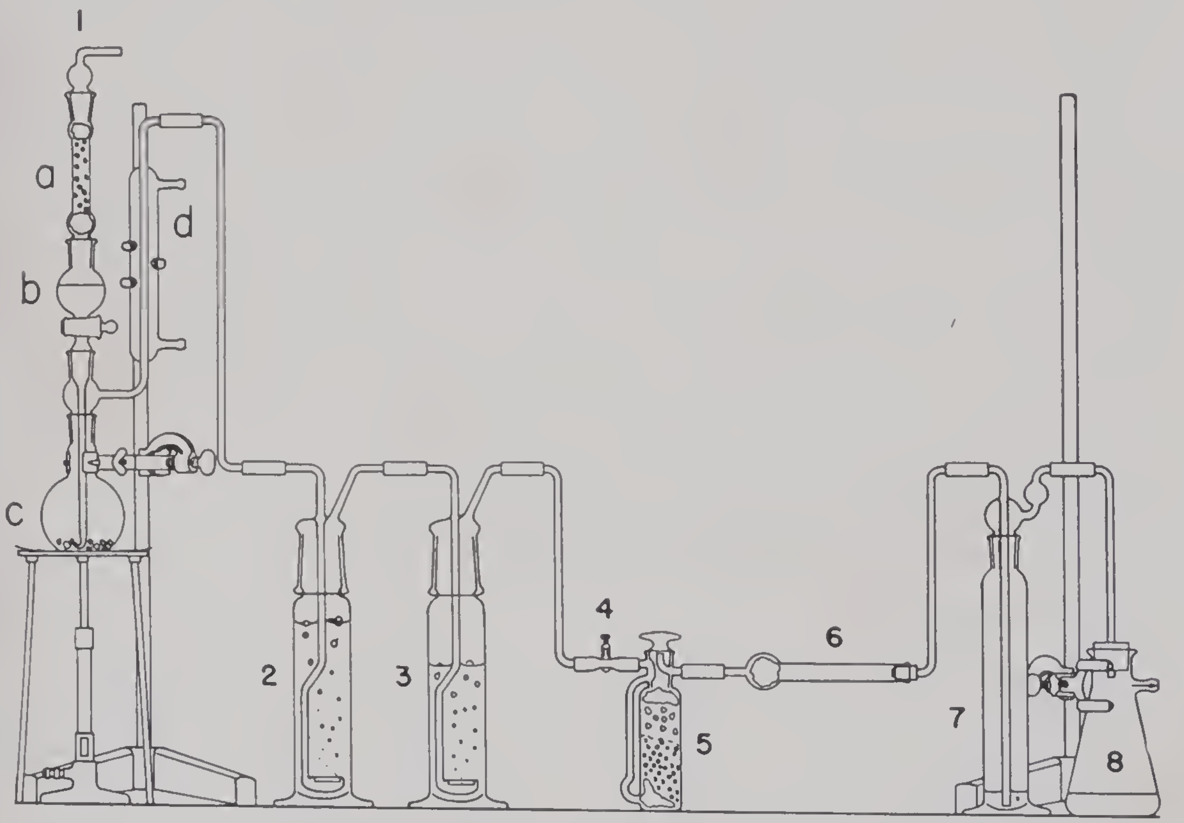
Remove bomb from the container and cool under the tap for 20 minutes to insure complete condensation of H_2SO_4. Release pressure slowly, open, and rinse contents of bomb and capsule with hot distilled water into a 400 ml. beaker. Measure the length of the unburned fuse wire and record. Titrate the solution with standard Na_2CO_3 solution, made so that 1 ml. of solution is equivalent to 1 cal. (3.658 gm. anhydrous Na_2CO_3 per liter), to obtain the correction value in calories for the oxidation of the portion of nitrogen which remained as free acid in the bomb after combustion. After titration, acidify the solution with 10 ml. of 10 per cent HCl and save for sulfur determination (see p. 269).

Energy in food and feces: Weigh 2 gm. of vacuum dried food or 1 gm. of dry feces (dried from the frozen state *in vacuo*) into an illium capsule and carry out oxygen bomb calorimeter procedure given for determination of energy in urine.

CARBON

The wet combustion method of macro carbon determination depends upon the oxidation of organic material by a potassium dichromate-sulfuric acid solution, with the consequent release of carbon dioxide. The carbon dioxide is collected by absorption with ascarite and weighed. The method is applicable to fresh urine, and to dried foods and feces.

The Knorr apparatus used in the combustion is shown in the figure. Carbon dioxide produced by the reaction is carried from the combustion vessel through a series of two scrubbers (2, 3) designed for the removal of water vapor and the variety of interfering substances (such as SO_2 and oxides of nitrogen) which arise on combustion of food and excreta. The sulfuric acid-



APPARATUS FOR DETERMINATION OF CARBON

1. Knorr apparatus (Scientific Glass Apparatus Co. #J885): (*a*) Drying tube containing 12–20 mesh ascarite (cotton retainers); (*b*) Dropping funnel with stopcock, containing sulfuric acid; (*c*) Combustion flask; (*d*) Condenser.
2, 3. Pyrex scrubbers containing chromate sulfuric acid solution. (Add concentrated H_2SO_4 to a saturated solution of $K_2Cr_2O_7$ until the precipitate which appears at first is redissolved.)
4. Screw clamp.
5. Nesbitt absorption bulb containing one-quarter inch layer of Dehydrite and ascarite to fill the flask.
6. Trap.
7. Bubble counter containing concentrated H_2SO_4.
8. Flask attached to water pump.

chromic acid solution in the scrubbers should be changed after every four or five determinations to maintain efficiency in its absorption properties. The washed carbon dioxide gas is then absorbed on ascarite. The gas stream flow is maintained by a vacuum water pump.

Procedure

Test the apparatus for leaks by closing stopcock on funnel (1*b*) and applying suction. After 10 minutes, less than one bubble per minute should be passing bubble counter (7). Place a sample containing 200 to 300 mg. carbon in the combustion flask (1*c*), together with 10 gm. $K_2Cr_2O_7$. Apply gentle suction and draw 50 ml. concentrated H_2SO_4 into the flask from the funnel (1*b*). One or two milliliters of H_2SO_4 should be allowed to remain above the stopcock as a seal. Maintain suction and heat the flask until foaming begins, then withdraw the flame until the reaction is complete. Heat the combustion flask strongly for five minutes to completely convert any remaining carbon to CO_2. The flow of gas should be such that not more than five bubbles per second pass the bubble counter. Remove the flame, gradually increase the suction to maintain a flow of gas, and admit CO_2-free air through the funnel. Sweep out the apparatus for 30 minutes, then weigh the absorption flask (5), using a counterpoise. Replace the flask on the line for another five-minute aspiration period and reweigh. Repeat this procedure until successive weighings agree within two milligrams.

MACRO CARBON DETERMINATION

Material analyzed: **Feces**

Subject:

Average daily Dry weight: **20.62**

Average daily Urine volume:

Balance period:

Date of analysis:

	SAMPLE I*	SAMPLE II	SAMPLE III
Weight of sample (gm.) Volume of sample (ml.)	**0.2113**		
Final weight of absorption bulb (gm.)	**15.4174**		
Initial weight of absorption bulb (gm.)	**15.0601**		
Weight of CO_2 absorbed (gm.)	**0.3573**		
Carbon in sample (gm.)	**0.09744**	(Weight of CO_2 absorbed $\times 0.2727$)	
Carbon per (gm.) (ml.) sample (gm.)	**0.4611**	(Carbon in sample ÷ weight or volume of sample)	
Carbon per day (gm.)	**9.51**	(Carbon per gm. or ml. sample × avg. daily dry weight or volume)	

* Illustrative values were calculated by machine. Some imply greater accuracy than the procedure warrants (See section on Treatment of Data).

FATS

The method for determination of the fat distribution in feces is based upon procedure given by Tidwell and Holt.*

Procedure

Foods: Weigh out a suitable sample (1 to 5 gm., inversely to the fat content) of vacuum dried food and place on No. 43, Whatman fat-free filter paper (15 or 18.5 cm.). Fold the paper envelope-fashion so that not any of the sample can escape; roll the folded paper and place in a Soxhlet extractor fitted to a weighed, 100 ml. receiving flask. Add approximately 60 ml. of anhydrous ethyl ether and extract 16 hours on a controlled water bath. (The amount of ether used is approximately 50 per cent more than is necessary to cause siphoning and allows for any evaporation which may occur.) Place receiving flask on steam bath and evaporate contents to dryness. Put the flask in a vacuum desiccator (metal) containing barium perchlorate (Desicchlora) and evacuate. The flask containing fat can be weighed after drying for two hours.

Feces: *Ether-soluble lipids:* Weigh 1 gm. of feces which has been dried from the frozen state onto No. 43, Whatman fat-free filter paper, insert in Soxhlet extractor and extract as described for food, except that a 150 ml. receiving flask should be used.

Ether-soluble lipid partition: The extract, which contains all of the fecal fat except that contained in the soaps, is partitioned into free fatty acids, unsaponifiable fat and neutral fat. The ether extracted feces are analyzed for fatty acids which are present in the form of soaps.

Free fatty acid: Add 50 ml. of absolute alcohol to the flask containing the extracted fat and warm on steam bath until the fatty substances are in solution. Add 10 drops of a one per cent solution of phenolphthalein in 95 per cent alcohol. Titrate the solution and a blank of 50 ml. of the alcohol, with standard aqueous 0.1 N NaOH. Each milliliter of N NaOH is equivalent to 0.268 gm. free fatty acid.

Unsaponifiable fat: After titration for the fatty acid, transfer contents of the flask into a 250 ml. flat-bottom flask, using small portions of alcohol, then ether, to complete the transfer. Add 0.1 ml. of a saturated solution of potassium hydroxide and 0.5 ml. of water and place flask on the steam bath. Evaporate the solution until no trace of alcohol can be detected; then add 5 ml. of water, followed by 30 ml. ethyl ether. (Extraction of the unsaponifiable material by the ether is not complete unless the supernatant soap solution of triglycerides and free fatty acids is clear. If the water solution of soaps is cloudy, an emulsion has formed which, usually, can be broken by allowing the flask to stand over night or placing in the refrigerator for several hours.)

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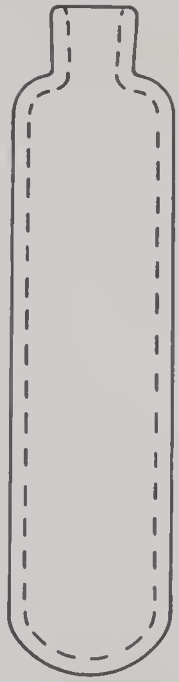
* Tidwell, Herbert C., and L. Emmett Holt, Jr., The estimation of the total lipids and the lipid partition in feces. *J. Biol. Chem.*, 112: 605, 1935. (Cf. Ref. 345).

Decant the ether solution containing the unsaponifiable fat fraction into a narrow mouth, round bottom, 50 ml. centrifuge tube (See Figure) containing 10 ml. of five per cent potassium hydroxide, stopper tightly with a cork and shake vigorously. After the ether has formed a layer, transfer it to a weighed, 250 ml. fat extraction flask, using a Van Slyke lipid transfer tube. Repeat the process of extracting the unsaponifiable fat from the soap solution three more times using 30 ml. portions of ethyl ether each time. Four extractions usually are sufficient but if the fourth ether extract is colored, further extraction is necessary. Place the weighed flask, containing the combined ether extracts, on the steam bath and evaporate the contents to dryness; evacuate in a desiccator for 30 minutes or longer and weigh the flask and unsaponifiable fat when cool.


Fifty milliliter centrifuge tube

Neutral fat: Add the values obtained by the titration for free fatty acids and by the extraction of the unsaponifiable portion. Subtract this sum from the total weight of fatty material extracted in the Soxhlet apparatus to obtain the weight of the triglyceride or "neutral fat" portion.

Soaps: The fact that feces contain fatty acids, combined in the form of soaps which are not extractable by fat solvents, is unique among biological materials. Remove the filter paper, containing the ether-extracted feces, from the Soxhlet extraction apparatus, and, after allowing the ether to evaporate, transfer the dry material to a dry, narrow mouth, round bottom, 50 ml. centrifuge tube. Add 2 ml. of concentrated HCl, followed by 3 ml. of water. Warm the tube 10 minutes in a steam bath, with intervals of shaking to insure a uniform mixture, and allow it to cool.

Fill the tube to within one inch of the top with a mixture of equal portions of ethyl and petroleum ether; stopper with a clean cork and shake vigorously. Centrifuge for five minutes and transfer the ether extract to a weighed, 250 ml., fat extraction flask, using a Van Slyke lipid transfer tube. Repeat the extraction process with the mixed ether three more times to insure complete extraction of the fatty acids derived from the soaps. Place the weighed flask containing the combined extracts on the steam bath and evaporate to dryness. Weigh after cooling in a desiccator. The weight of the fatty acids derived from the soaps, multiplied by 1.1, gives the weight of the soaps.

Total fat: The total fat content of the feces can be calculated by totaling the weights of the different fractions.

FAT CONTENT OF FOOD

Diet: Date of analysis: Balance period:

	SAMPLE I*	SAMPLE II	SAMPLE III
Dry weight of sample (gm.)	2		
Weight of flask + fat (gm.)	58.7821		
Weight of flask (gm.)	58.5496		
Weight of fat in sample (gm.)	0.2325		
Weight of fat per gram dry sample (gm.)	0.11625	(Fat in sample ÷ dry weight of sample)	
Average daily dry weight of food† (gm.)	282		
Fat intake per day (gm.)	32.78	(Fat per gm. dry wt. of sample × avg. daily dry weight of food intake)	

* Illustrative values were calculated by machine. Some imply greater accuracy than the procedure warrants (See section on Treatment of Data).

† From record of preparation of composite for analysis (p. 235).

FAT CONTENT OF FECES

Balance period:

Subject:

Average daily fecal dry weight:* 20.8

Date of analysis:

	SAMPLE I	SAMPLE II	SAMPLE III
Dry weight of sample (gm.)	1		
Weight of flask +ether-soluble fat (gm.)	66.1426		
Weight of flask (gm.)	66.0843		
Weight of ether soluble fat (gm.)	0.0583		
Weight of ether soluble fat per gram dry sample (gm.)	0.0583	(Ether soluble fat in sample ÷dry weight of sample)	
Ether soluble fat per day (gm.)	1.213	(Ether soluble fat per gram sample ×average daily fecal dry weight)	
NaOH used in titration of sample (ml.)	0.59		
NaOH used in titration of blank (ml.)	0.1		
NaOH neutralized by free fatty acid (ml.)	0.49		
Normality of NaOH	0.08865		
Normal NaOH equivalent neutralized by free fatty acids (ml.)	0.04344		
Free fatty acid in sample (gm.)	0.0116	(Normal NaOH used ×0.268)	
Free fatty acid per gram dry sample (gm.)	0.0116	(Free fatty acid in sample ÷dry weight of sample)	
Free fatty acid per day (gm.)	0.241	(Free fatty acid per gram sample × average daily fecal dry weight)	
Weight of flask +unsaponifiable lipid (gm.)	69.6959		
Weight of flask (gm.)	69.6713		
Weight of unsaponifiable lipid in sample (gm.)	0.0246		
Weight of unsaponifiable lipid per gram sample (gm.)	0.0246	(Unsaponifiable lipid in sample ÷ dry weight of sample)	
Weight of unsaponifiable lipid per day (gm.)	0.512	(Unsaponifiable lipid per gm. sample ×avg. daily fecal dry weight)	
Neutral fat in sample (gm.)	0.0221	(Ether soluble fat — free fatty acid and unsaponifiable fat in sample)	
Neutral fat per gram dry sample (gm.)	0.0221	(Neutral fat in sample ÷dry weight of sample)	
Neutral fat per day (gm.)	0.460	(Neutral fat per gram sample × average daily fecal dry weight)	
Weight of flask +fatty acids from soaps (gm.)	73.7318		
Weight of flask (gm.)	73.6642		
Weight of fatty acids from soaps (gm.)	0.0676		
Soaps in sample (gm.)	0.0744	(Fatty acid from soap in sample ×1.1)	
Soaps per gram dry sample (gm.)	0.0744	(Soap in sample ÷dry weight of sample)	
Soaps per day (gm.)	1.548	(Soap per gram sample ×average daily fecal dry weight)	
Total fecal fat (gm./day)	2.761	(Sum of ether soluble fat and soap)	

* From record of preparing feces for analysis (p. 240).

LIGNIN, CELLULOSE AND HEMICELLULOSE

Lignin, cellulose and hemicellulose in food and feces are determined by adaptations of the methods of Williams and Olmsted and Shaffer and Somogyi.[*] Owing to the length of the procedure and the transfers involved it is desirable to run triplicate samples. The procedure is empirical and comparable results may be obtained only with highly standardized technique.

Procedure

Preparation of Indigestible Vegetable Residue

Foods: Extract 5 gm. dried food (dried at 40° C. *in vacuo* and ground to pass a 20 mesh sieve) with ether in a Soxhlet apparatus for eight hours. Dry *in vacuo,* and weigh 0.5 gm. of the fat-free material into a 125 ml., glass-stoppered Erlenmeyer flask. Add 50 ml. water and autoclave for 30 minutes at 15 pounds pressure, with the stopper removed from the flask. Cool below 50° C. and add 40 ml. buffer bile salt solution,[†] 10 ml. pancreatin-sodium chloride,[‡] and a few drops of toluene. Stopper, mix well, and incubate at 38.5° C. for three days.

After incubation, filter through bolting silk, adding the digest slowly with an accompanying stream of hot water from a wash bottle. Wash the residue with at least 200 ml. of hot distilled water; then, successively, with 50 ml. of hot ethyl alcohol, 25 ml. of hot benzene, and 25 ml. of ethyl ether. Transfer the residue to a 50 ml., glass-stoppered Erlenmeyer flask and dry in an oven at 70° C. for two hours. (The transfer is best effected just after the residue becomes crumbly, and before the ether is completely evaporated.)

Feces: Weigh 0.25 gm. of feces which have been dried under vacuum from the frozen state, into a 125 ml., glass-stoppered Erlenmeyer flask. Add 25 ml. distilled water, and autoclave

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[*] Williams, Ray D., and W. H. Olmsted. A biochemical method for determining indigestible residue (crude fiber) in feces: lignin, cellulose and non-water soluble hemicelluloses, *J. Biol. Chem.*, 108: 653, 1935. (cf. Ref. 387)

Shaffer, Philip A., and Michael Somogyi. Copper-iodometric reagents for sugar determination. *J. Biol. Chem.*, 100: 695, 1933. (cf. Ref. 302)

[†] Buffer bile salt solution: To 50 ml. of 0.2 N potassium acid phosphate (27.23 grams of anhydrous KH_2PO_4 per liter) add 23.4 ml. of 0.4 N sodium hydroxide (16.0 grams per liter), 6.6 ml. distilled water and 2.0 gm. sodium taurocholate, Merck (pH 8 on final dilution). Solution will keep one week in refrigerator; discard if mold appears.

[‡] Pancreatin-sodium chloride solution: Add 10 gm. pancreatin (Merck's U.S.P. or 5 gm. Merck's absolute) to 100 ml. 8.5 per cent sodium chloride. Stir frequently for 30 minutes and filter through a medium paper. Make fresh daily.

(unstoppered) for 30 minutes. Cool below 50° C. and add 20 ml. buffer bile salt solution, 5 ml. pancreatin-sodium chloride solution, and a few drops of toluene. Stopper, mix well and incubate for three days at 38.5° C. Filter through bolting silk, wash and dry residue as directed for foods. This indigestible residue preparation may stand, if perfectly dry, until it is convenient to proceed with the rest of the determination.

Analysis of Indigestible Vegetable Residue

Determination of lignin: Add 20 ml. of chilled (6–10° C.) 21.4 *N* H_2SO_4* to the indigestible residue of food or feces in a glass-stoppered container; shake briskly, and place in ice box (6–10° C.). Shake at hour intervals (particularly for the first five hours) and keep at this temperature for 24 hours.

Rinse the acid digest mixture into a 500 ml., round-bottom distilling flask. Dilute rapidly to 300 ml. with distilled water.

LIGNIN

Analyzed material: **Feces**

Subject:

Average daily dry weight: **23.43**

Balance period:

Date of analysis:

	SAMPLE I	SAMPLE II	SAMPLE III
Dry weight of sample (gm.)	**0.25**		
Weight of crucible + lignin (gm.)	**21.1406**		
Weight of crucible after ignition (gm.)	**21.1300**		
Weight of lignin in sample (gm.)	**0.0106**		
Lignin per gram sample (gm.)	**0.0424**	(Lignin in sample ÷ dry weight of sample)	
Lignin per day (gm.)	**0.993**	(Lignin per gram dry weight × average daily dry weight)	

Place the flask on a sandbath under a reflux condenser, and maintain boiling temperature for three hours. Cool to room temperature; filter into a 500 ml., pyrex suction flask through a loose layer of ignited asbestos (Gooch crucible) and wash thoroughly with water, adding the first 50 ml. of washings to the filtrate. Retain the filtrate and first washings for the determination of simple sugars from cellulose and hemicellulose. Wash the residue with at least 100 ml. of water, then with 30 ml. portions, successively, of alcohol, benzene and ether. Dry the residue at 110° C. for one-half hour, cool, and weigh. Ignite at 750° C. in a muffle furnace for one-half hour; cool; re-weigh, and calculate the loss as lignin.

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* Sulfuric acid, 21.4 *N*: Add 600 ml. 95 per cent H_2SO_4 (sp. gravity 1.84) to 400 ml. distilled water, with constant stirring. Cool, dilute to 1 liter and store in refrigerator.

Determination of cellulose and hemicellulose: Cellulose and hemicellulose are determined as sugar in the filtrate obtained from the lignin determination. Neutralize the filtrate to methyl red-methylene blue indicator with fifty per cent sodium hydroxide (it is important that this neutralization be exact). Transfer to a 500 ml. volumetric flask and dilute to volume when cool. Cellulose on hydrolysis yields the fermentable sugar, glucose. Hemicellulose yields the unfermentable pentose sugars, arabinose and xylose. Therefore, to differentiate between cellulose and hemicellulose, reducing sugar is determined in fermented and unfermented aliquots of the hydrolysate.

CELLULOSE

Analyzed material: **Feces**
Subject:
Average daily dry weight: **21.72**
Balance period:
Date of analysis:

	SAMPLE I*	SAMPLE II	SAMPLE III
Dry weight of sample (gm.)	**0.25**		
Volume of diluted digest (ml.)	**500**		
Aliquot taken (ml.)	**10**		
Volume $Na_2S_2O_3$ for sugar titration of *fermented* aliquot (ml.)	**9.00**		
Volume $Na_2S_2O_3$ for sugar titration of *unfermented* aliquot (ml.)	**8.03**		
Volume $Na_2S_2O_3$ for titration of glucose (ml.)	**0.97**	(Volume for titration of fermented −volume for unfermented aliquot)	
Glucose in aliquot (mg.)	**0.173**	(From standard curve)	
Glucose per ml. of aliquot (mg.)	**0.0173**	(Glucose in aliquot ÷volume of aliquot)	
Glucose in sample (mg.)	**8.65**	(Glucose per ml. ×volume of diluted digest)	
Glucose per gram dry weight (mg.)	**34.60**	(Glucose in sample ÷dry weight of sample)	
Cellulose per gram dry weight (mg.)	**31.14**	(Glucose in sample ×0.9)	
Cellulose per day (mg.)	**676**	(Cellulose per gm. dry weight ×average daily weight)	

* Illustrative values were calculated by machine. Some imply greater accuracy than the procedure warrants (See section on Treatment of Data).

Centrifuge 7 ml. of yeast suspension[*] in a 50 ml. heavy walled centrifuge tube for 10 minutes and drain on filter paper. Add 15 ml. of the filtrate from the lignin determination, mix and incubate in oven (38.5° C.) for 30 minutes; stir every five minutes. Centrifuge fermented solution for 10 minutes and carefully pour the supernatant liquid into a clean, dry test tube.

Measure accurately, 10 ml. portions of fermented and unfermented solutions into pyrex test tubes (25×200 mm.). (The total volume of sugar solution and reagent should always be identical with the volumes of standard solutions used in determining the calibration curve.) Add exactly 5 ml. of copper-iodometric reagent[†] to each so that the sugar solutions are rinsed from the walls of the test tubes. At the same time, measure 5 ml. portions of the reagent for blank determinations, adding 10 ml. water instead of sugar solution. Mix the solutions with gentle shaking and cover the tubes with glass balls just large enough to be supported on the rims of the test tubes. Place the tubes in metal racks which hold them securely, and which fit into a water bath. The bath should be boiling vigorously at the time the tubes are introduced. Boil for 30 minutes (use interval timer) and remove rack and tubes to a pan of cold water. When cool, add to each tube, 5 ml. N H_2SO_4, mix well to dissolve *completely* all of the cuprous iodide precipitate, and allow to stand (covered) for 5 to 10 minutes. Rinse the glass balls and walls of tubes and titrate with 0.005 N sodium thiosulfate,[§] determining the end-point by using soluble starch indicator.* *If the titration requires less than 1 ml. of sodium thiosulfate the result*

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[*] Washed yeast suspension: Place one-half cake Fleischmann's compressed yeast (about 6 gm.) in a 50 ml. heavy walled centrifuge tube. Add 25 ml. water, stir contents and centrifuge for 8 to 10 minutes at 1800 R.P.M. Pour off supernatant liquid and repeat washing procedure four times. (The washed yeast may be stored in the refrigerator for two or three days.) When ready to use, add 25 ml. water, stir until a uniform suspension is formed, and pipette.

[†] Copper-iodometric reagent: Dissolve 25 gm. anhydrous sodium carbonate (Na_2CO_3) and 25 gm. of Rochelle salt in 500 ml. of water. Add 75 ml., 10 per cent copper sulfate ($CuSO_4 \cdot 5H_2O$) from a pipette extending well below surface of the liquid, stirring constantly. Add 20 gm. dry sodium bicarbonate ($NaHCO_3$) and dissolve by stirring; then add 5 gm. potassium iodide. Rinse into one liter volumetric flask and add 50 ml. standard potassium iodate solution.[‡] Dilute to the mark, mix, and filter through No. 42 Whatman filter paper. Store in pyrex, glass-stoppered bottle and protect from strong light. Keeps over a year.

[‡] Standard potassium iodate solution: Dissolve 3.567 gm. pure KIO_3 in one liter of water. (When treated with an excess of KI and H_2SO_4, solution liberates iodine equivalent to its volume of 0.1 N.)

[§] Sodium thiosulfate solution, 0.005 N: Calculate from the normality factor of the 0.1 N solution the amount required in one liter of exactly 0.005 N solution. Add 10 ml. of 0.1 N NaOH before dilution, to improve stability. Dilute to one liter. Solution maintains its titer for several days. Standardize against 10 ml., 0.01 N KIO_3 (dilute standard potassium iodate 1:10) to which 1 ml. N

HEMICELLULOSE*

Analyzed material: **Feces**
Average daily dry weight: **21.72**

Subject:
Balance period:
Date of analysis:

	SAMPLE I	SAMPLE II	SAMPLE III
Dry weight of sample (gm.)	**0.25**		
Volume of diluted digest (ml.)	**500**		
Aliquot taken (ml.)	**10**		
Volume $Na_2S_2O_3$ for titration of blank (ml.)	**9.92**		
Volume $Na_2S_2O_3$ for titration of fermented aliquot (ml.)	**9.00**		
Volume $Na_2S_2O_3$ for titration of pentose (ml.)	**0.92**	(Volume for blank − volume for fermented aliquot)	
Pentose in aliquot (mg.)	**0.153**	(From standard curve)	
Pentose per ml. of aliquot (mg.)	**0.0153**	(Pentose in aliquot ÷ volume of aliquot)	
Pentose in sample (mg.)	**7.65**	(Pentose per ml. of aliquot × volume of diluted sample)	
Pentose per mg. dry weight (mg.)	**30.60**	(Pentose in sample ÷ dry weight in sample)	
Hemicellulose per gram dry weight (mg.)	**26.93**	(Pentose per gram dry weight ×0.88)	
Hemicellulose per day (mg.)	**585**	(Hemicellulose per gm. dry weight ×daily dry weight)	

* The analytical values obtained in determination of total hemicellulose may be recorded on the form used for "hemicellulose."

is of doubtful significance because the capacity of the reagent is approached. Repeat the determination with diluted sugar solution.

In order to calculate the sugar values for cellulose and hemi-

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H_2SO_4 and 2 ml., 2.5 per cent potassium iodide have been added. This amount should titrate *exactly* 20 ml. of 0.005 *N* sodium thiosulfate solution. Five milliliters of copper-iodometric reagent (equivalent to 0.005 *N* iodate) should titrate exactly 5 ml. of 0.005 *N* sodium thiosulfate under the same conditions.

Sodium thiosulfate solution, 0.1 *N*: Dissolve 25 gm., $Na_2S_2O_3 \cdot 5H_2O$ in water; add 10 ml. 0.1 *N* NaOH and dilute to one liter. Standardize by titration against standard potassium iodate solution: To 25 ml. potassium iodate solution add 50 ml. water, 1 gm. KI and 5 ml. *N* H_2SO_4; titrate to a pale straw color with thiosulfate solution; add 1 ml. soluble starch solution (see footnote*) and titrate to disappearance of the blue color.

* Soluble starch solution: Heat 100 ml. saturated benzoic acid (5 gm. per liter will maintain saturation) to near boiling. Mix 1 gm. soluble starch with a little water and add to the hot benzoic acid. This solution keeps about one week.

cellulose, standard titration curves are prepared for glucose* and a mixture of xylose and arabinose.† The copper iodometric reagent is calibrated with standard sugar solutions under the exact conditions used in the analysis of unknown materials. Dilutions of standard sugar solution containing 0.1 to 1.0 mg. of sugar in 10 ml. are analyzed and the milligrams of sugar *vs* titration in milliliters are plotted on graph paper. The values should have a linear relationship. Interpret the difference in titration of the blank and the fermented aliquot on the xylose-arabinose curve, and multiply the apparent milligrams of xylose and arabinose by 0.88, the conversion factor for pentose to pentosan (hemicellulose). Interpret the difference in titration of the fermented and the unfermented aliquots on the glucose curve, and multiply by 0.90, the conversion factor for glucose to cellulose.

Total hemicellulose of food: Since some of the hemicellulose in fruits and vegetables is soluble at *pH* 8 during pancreatin digestion, total hemicellulose is determined by a modified method.

Weigh 0.5 gm. of dry, fat-free food into a 125 ml. Erlenmeyer flask; autoclave for 30 minutes at 15 pounds pressure, and cool. Filter the residue through bolting silk, wash with hot distilled water, followed by hot alcohol, hot benzene and ether. Transfer residue to a 50 ml., glass-stoppered Erlenmeyer flask, and dry for two hours at 70° C. Cool; add 20 ml. cold, 21.4 *N* H_2SO_4 and keep at 6 to 10° C. for 24 hours, with occasional shaking (at least every hour for the first five hours). Rinse into a 500 ml. flask and dilute rapidly with water to 300 ml. Keep near the boiling point for three hours, cool and filter off undigested residue, collecting the filtrate and 50 ml. washings (water) in a 500 ml. suction flask. Discard the residue and neutralize the filtrate to methyl red-methylene blue with 50 per cent sodium hydroxide. Transfer quantitatively to a 500 ml. volumetric flask, and make to volume when the solution is at room temperature. Ferment 15 ml. aliquots with washed yeast, and determine unfermentable sugar as directed before. Interpret the results on the xylose-arabinose curve and calculate as total hemicellulose. The difference between the total hemicellulose of foods, and the hemicellulose determined when pancreatin digestion is used (stable hemicellulose), is designated as *labile* hemicellulose.

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[*] Standard glucose solution: Weigh one gram pure glucose into a one liter volumetric flask. Make to volume with 0.85 *N* sodium sulfate solution (60.38 gm. Na_2SO_4 per liter).

[†] Standard xylose-arabinose solution: Weigh 500 mg. xylose and 500 mg. arabinose into the same one liter volumetric flask, and make to volume with 0.85 *N* sodium sulfate solution.

CALCIUM

The method for determination of calcium is based upon the procedure given by Scott.*

Procedure

Urine: If undiluted urine is alkaline to litmus, make acid with a few drops of concentrated HCl and take 200 ml. for analysis. (If a precipitate is present in the acidified urine, filter through a dry paper and take urine samples from the filtrate.) Add one drop of methyl red-methylene blue indicator and add concentrated NH_4OH until solution just turns alkaline. Add 10 per cent acetic acid, dropwise, until the solution is slightly acid; then add 10 ml. additional. Heat to boiling and add 10 ml. of five per cent ammonium oxalate, stirring constantly; let stand at least four hours. Filter through ash-free paper into a large porcelain casserole, wash the beaker and the precipitate seven times with hot 0.5 per cent ammonium oxalate (until precipitate is free of chlorides) and twice with distilled water (to free precipitate of ammonium oxalate); then fill the paper cone completely with a final washing of water. (Retain combined filtrate and washings for magnesium determination.)

Place the original beaker under the filter funnel and dissolve any precipitate by filling the filter paper cone with hot, N H_2SO_4. If there are no urate salts in the precipitate, indicated by the absence of any yellow or brown color, the paper filter may be punctured and the precipitate washed into the beaker with hot acid. Add 2 to 3 ml. concentrated H_2SO_4 to the solution, and titrate at 60 to 80° C. with $0.05\,N$ $KMnO_4$.† The titration end point is reached when the pink color of $KMnO_4$ persists 15 to 20 seconds.

Foods, feces: To a suitable aliquot of the ash solution (10 gm. foods, 5 gm. feces) add 8 to 10 drops concentrated HNO_3 and heat to boiling. Rinse down the sides of the beaker with water, diluting to a volume of 150 ml. Add five drops of 10 per cent citric acid, and add concentrated NH_4OH until the first trace of permanent precipitate appears (hydroxides of aluminum and iron). Just dissolve this precipitate by adding 10 per cent acetic acid, then add 10 ml. more of the acetic acid solution. Heat to boiling and add 10 ml. of five per cent ammonium oxalate solution, stirring constantly. Let stand at least four hours; then filter and continue the procedure as outlined for urine.

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* Scott, W. W. Standard Methods of Chemical Analysis, Volume I, The Elements. 4th Ed., p. 108. D. Van Nostrand Co. Inc., New York, 1927. (cf. Ref. 297)

† Tenth normal solution potassium permanganate (3.162 gm. per liter). Dilute to 0.05 N and standardize with 0.05 N sodium oxalate, freshly prepared from 0.1 N sodium oxalate. One milliliter 0.05 N $KMnO_4$ is equivalent to 1 mg. calcium. One milliliter of N $KMnO_4$ is equivalent to 0.02 gm. calcium.

CALCIUM

Analyzed material: **Feces**

Subject:

Average daily Volume (ml.):
Average daily Dry weight (gm.): **20.8**

Balance period:

Date of analysis:

Volume (ml.) / Dry weight (gm.) of sample taken	SAMPLE I*	SAMPLE II	SAMPLE III
Volume (ml.) Dry weight (gm.)	**10**		
Ash diluted to (ml.)	**100**		
Aliquot taken (ml.)	**25**		
Dry weight in aliquot (gm.)	**2.5**		
Volume of $KMnO_4$ used (ml.)	**89.26**		
Normality of $KMnO_4$	**0.0500**		
Normal $KMnO_4$ equivalent (ml.)	**4.4630**		
Calcium in aliquot (gm.)	**0.089260**	(Normal $KMnO_4$ equivalent ×0.02)	
Calcium per ml. urine (gm.) Calcium per gm. dry wt. (gm.)	 **0.035704**	(Calcium in aliquot ÷average daily urine volume or dry weight)	
Calcium per day (gm.)	**0.743**	(Calcium per ml. urine or gm. dry wt. ×avg. daily volume or dry wt.)	

* Illustrative values were calculated by machine. Some imply greater accuracy than the procedure warrants (See section on Treatment of Data).

MAGNESIUM

The method for determination of magnesium is based upon the method given by Willard and Furman.*

Procedure

Add to the filtrate from the calcium determination, in the casserole, 20 ml. concentrated HNO_3 and 7 ml. concentrated HCl and evaporate to dryness. Repeat. Heat the casserole to redness over a Meeker burner until all the organic material has been burned off and the ammonium salts destroyed. Cool, dissolve the residue in concentrated HCl; warm slightly; dilute with 20 ml. distilled water and filter into an unscratched beaker. Wash the filter paper with water until the filtrate has a volume of 85 to 90 ml. Then add 10 ml. of ammonium phosphate reagent,† three drops alizarine indicator (0.1 per cent in water), and, with foods and feces samples, add 5 ml. ammonium tartrate solution.‡ Make just alkaline with concentrated NH_4OH, then add *slowly, with constant stirring* dilute NH_4OH (1:7) to increase the volume of the solution by one-fourth to one-third. Let stand two hours (or over night) and filter through a weighed, porous porcelain crucible (Gooch). Wash free of chlorides with alcoholic ammonia.§ Dry and ignite for one-half hour at bright red heat (1000° C.); cool; place in desiccator and weigh the magnesium pyrophosphate ($Mg_2P_2O_7$).

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* Willard, Hobart H., and N. Howell Furman. Elementary Quantitative Analysis. Theory and Practise, 2nd Ed., p. 353. D. Van Nostrand Co., Inc., New York, 1935. (cf. Ref. 381)

† Reagent: Dissolve 10 gm. ammonium chloride and 10 gm. ammonium dihydrogen phosphate ($NH_4H_2PO_4$) in distilled water to make 100 ml. of solution.

‡ Ammonium tartrate solution: Dissolve 20 gm. ammonium tartrate in 100 ml. distilled water. Ammonium tartrate solution is added to keep in solution the iron and aluminum present in considerable quantities in foods and feces. The ammonium tartrate added must be sufficient in amount to prevent precipitation of the iron and aluminum, but must not be in great excess of the required amount, for too high concentration may prevent complete precipitation of the magnesium. With the materials sampled in our investigation, 5 ml. of the above ammonium tartrate solution were ample to keep the heavy metals in solution. (Iron, manganese and aluminum were not found in the precipitates when examined polarographically.) With samples containing other relative amounts of iron and aluminum the concentration of ammonium tartrate must be adjusted to provide the quantity needed to obtain precipitates free of contamination.

§ Alcoholic ammonia: One part 95 per cent alcohol; one part dilute (1:2) NH_4OH; three parts water.

MAGNESIUM

Analyzed material: **Feces**

Subject:

Average daily volume (ml.):

Average daily dry weight (gm.): **20.8**

Balance period:

Date of analysis:

Volume / Dry weight of sample (ml.) / (gm.)	SAMPLE I*	SAMPLE II	SAMPLE III
	2.5	(From calcium determination)	
Weight of dish + $Mg_2P_2O_7$ (gm.)	**16.7186**		
Weight of dish (gm.)	**16.6120**		
Weight of $Mg_2P_2O_7$ (gm.)	**0.1066**		
Magnesium in sample (gm.)	**0.02328**	($Mg_2P_2O_7$ in sample ×0.2184)	
Magnesium per ml. urine (gm.) Magnesium per gm. dry weight (gm.)	 **0.009312**	(Magnesium in sample ÷ volume or dry weight in aliquot)	
Magnesium per day (gm.)	**0.1937**	(Magnesium per gram dry sample ×average daily dry weight)	

* Illustrative values were calculated by machine. Some imply greater accuracy than the procedure warrants (See section on Treatment of Data).

POTASSIUM

The method for the determination of potassium is based upon the method of Kramer and Tisdall.*

Procedure

Urine: Measure 2 ml. portions of dilute urine (1:10) containing 0.2 to 0.5 mg. of potassium, into 15 ml., graduated, pyrex centrifuge tubes; add three drops concentrated HNO_3 and one drop concentrated HCl and evaporate to dryness in the oven at 110° C. Take up residue in 1 ml. 0.1 *N* HCl; then add 0.5 ml. of sodium nitrite solution;† mix and let stand five minutes. Add water to make the volume 4 ml. and mix; then add, drop by drop with constant shaking, 2 ml. of sodium cobaltinitrite reagent.‡ (It is particularly important that all samples be precipitated at the same rate of speed and, as a control, that 1 ml. of a standard solution of potassium§ be run with each set of unknowns, omitting digestion with concentrated hydrochloric and nitric acids.)

Let stand about three-quarters of an hour; then centrifuge seven minutes at approximately 1300 r.p.m. Remove all but 0.2 to 0.3 ml. of the supernatant liquid with a siphon tube attached to the suction pump. (The syphon tube is made by drawing a piece of glass tubing to a capillary about 1 mm. in diameter and bending the fine tip back in "U" form.) Wash four times; each time run 5 ml. of water down the sides of the tube so that the water is mixed with the residual fluid without disturbing the precipitate, and centrifuge for five minutes. The supernatant fluid from the last washing should be perfectly clear.

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* Kramer, B., and F. Tisdall. A clinical method for quantitative determination of potassium in small amounts of serum. *J. Biol. Chem.*, 46: 339, 1921. (cf. Ref. 189)

† Sodium nitrite solution: Fifty grams of potassium-free Merck's "reagent" sodium nitrite in 100 ml. of water.

‡ Sodium cobaltinitrite reagent: (a) Dissolve 25 gm. of cobalt nitrate crystals in 50 ml. of water and add 12.5 ml. of glacial acetic acid. (b) Dissolve 120 gm. of sodium nitrite (potassium-free, Merck) in 180 ml. of water. Add 210 ml. of (b) to all of (a) and bubble air through the solution until all the nitric oxide gas has been driven off. (Reagent will keep for a month in the refrigerator. Filter before using.)

§ Standard potassium solution: Standard solutions should be made up containing approximately the same Na:K ratio as the samples to be analyzed; for food and urine, 1 gm. KH_2PO_4 and 0.5 gm. NaCl are dissolved in water and diluted accurately to 1 liter; for feces, 1 gm. KH_2PO_4 and 0.20 gm. NaCl are diluted to 1 liter; 1 ml. of each of these solutions gives a satisfactory amount for the determination of the factor for calculation. *Known* determinations are run on mixtures of KH_2PO_4 plus NaCl, containing approximately the same Na:K ratio as the sample to be analyzed.

Add an excess of standard 0.02 *N* potassium permanganate* (about 2 ml. usually), then 1 ml. 4 *N* H_2SO_4 and mix thoroughly with a glass rod. Heat sample in boiling water bath (approximately one minute) until the solution becomes clear but remains pink (if it decolorizes, quickly add another 1 ml. $KMnO_4$.) *Do not heat too long.* If all the precipitate is not oxidized, the contents will be cloudy and the intensity of the color will diminish.

POTASSIUM

Analyzed material: **Feces**

Average daily $\left\{\begin{matrix}\text{volume (ml.):}\\\text{dry wt. (gm.): } \mathbf{20.8}\end{matrix}\right.$

Subject:

Balance period:

Date of analysis:

	SAMPLE I	SAMPLE II	SAMPLE III
Volume of urine ash solution in sample (ml.)	1		
Dry weight in sample (gm.)	0.02		(Foods and feces only)
Oxalate added (ml.)	1		
Normality of oxalate	0.01		
Normal oxalate equivalent (ml.)	0.01		
$KMnO_4$ added (ml.)	3		
$KMnO_4$ used in titration (ml.)	0.225		
$KMnO_4$ used (ml.)	3.225		($KMnO_4$ added + $KMnO_4$ used in titration)
Normality of $KMnO_4$	0.0198		
Normal $KMnO_4$ equivalent (ml.)	0.06386		
Normal $KMnO_4$ equivalent to potassium in sample (ml.)	0.05386		(Normal $KMnO_4$ equivalent − normal oxalate equivalent)
Potassium in sample (mg.)	0.3514		(Normal $KMnO_4$ excess × conversion factor* 6.524)
Potassium per $\left\{\begin{matrix}\text{ml.}\\\text{gram}\end{matrix}\right\}$ sample (mg.)	17.57		(Potassium in sample ÷ volume or dry weight in sample)
Potassium per day (mg.)	365		(Potassium per gram sample × avg. daily volume or dry weight)

* The conversion factor representing the milligrams of potassium indicated by 1 ml. *N* $KMnO_4$ in titration should be determined by each analyst under the conditions he employs, as:

$$\frac{\text{Milligrams potassium per milliliter of standard potassium solution}}{\text{Milliliters standard potassium permanganate used}}.$$

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* Potassium permanganate solution, 0.02 *N*: Make by diluting 0.1 *N* $KMnO_4$ and standardize daily against 0.01 *N* sodium oxalate.

Heating should be continued until the solution is clear but still pink. When the heating is continued too long, the contents again become cloudy and have a brownish color. If this is allowed to happen, the sample must be discarded as high results will be obtained. As soon as the solution becomes clear, promptly add an amount of standard 0.01 *N* sodium oxalate (usually 2 ml.) sufficient to decolorize the excess permanganate in the solution. The excess of oxalate is then titrated to a faint pink endpoint (persisting 15 seconds) with 0.02 *N* $KMnO_4$, delivered from a microburette graduated at 0.02 ml. intervals.

Foods and feces: Analyze the ash solutions as described for urine, selecting a dilution which contains from 0.2 to 0.5 mg. of potassium in a volume of 1 ml.

SODIUM

Sodium is determined by the Butler and Tuthill application of the gravimetric method of Barber and Kolthoff.[*]

Procedure

Urine: To approximately 10 ml. of urine (containing 1 to 10 mg. of sodium) in a 50 ml., glass-stoppered Erlenmeyer flask, add one drop phenolphthalein solution[†] and 0.2 gm. powdered calcium hydroxide. Shake and let stand 30 minutes with occasional shaking. The solution should turn pink. Filter through quantitative filter paper into a dry beaker and pipette 2 ml. of the filtrate into approximately 20 ml. of uranyl zinc acetate reagent[‡] in a 100 ml. beaker; stir constantly and for at least one minute after a precipitate appears. Cover the beaker with a watch glass and set aside for one hour. Transfer quantitatively into a weighed, porous porcelain crucible (Gooch) making the transfer of salt to crucible with two 5 ml. portions of uranyl zinc acetate reagent, then four 5 ml. portions of 95 per cent alcohol saturated with the triple salt, sodium uranyl zinc acetate, (filter *both* solutions before using). Finally, wash with two 5 ml. portions of ether. Apply suction until the precipitate is thoroughly dry, place in desiccator and weigh after several hours or over night. A blank should be run on a volume of distilled water equivalent to sample to make certain that the reagents are sodium-free.

Foods: Pipette 10 ml. of ash solution (containing 1 to 10 mg. Na) into a 25 ml. volumetric flask. Add one drop phenolphthalein and 0.2 gm. powdered calcium hydroxide. If solution is not alkaline, add concentrated NH_4OH, drop by drop, until the indicator turns pink. Make to volume with water, shake, and let stand 30 minutes. Filter through ash-free filter paper and pipette an aliquot of the filtrate (10 to 20 ml.) into a 100 ml. beaker. Evaporate to a volume of approximately 2 ml. on a steam bath. (If the evaporation is carried to dryness, the residues may be dissolved in 2 ml. of water containing a drop or two of concentrated HCl.) Then add 20 ml. of uranyl zinc acetate reagent to the solution, slowly and with constant stirring. Proceed through filtration, washing, and weighing as with urine.

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[*] Butler, A. M., and E. Tuthill. An application of the uranyl zinc acetate method for the determination of sodium in biological material. *J. Biol. Chem.*, 93, 171, 1931. (cf. Ref. 42)

[†] One per cent alcoholic solution of phenolphthalein.

[‡] Uranyl zinc acetate reagent: Solution A: 320 gm. sodium-free uranyl acetate, 58 gm. glacial acetic acid, add water to make 2080 gm. Solution B: 880 gm. zinc acetate, 29 gm. glacial acetic acid; add water to make 2080 gm. Cover and warm both solutions with occasional stirring until solution is complete. Mix while hot and let stand 24 hours. If no yellow precipitate appears, add 0.2 gm. of sodium uranyl zinc acetate $[(UO_2)_3ZnNa(CH_3COO)_9 \cdot 6H_2O]$ to saturate the solution. Shake occasionally, and filter any desired quantity immediately before using.

Feces: Pipette 25 ml. of ash solution (containing 1 to 10 mg. of sodium) into a 50 ml. volumetric flask. Add one drop phenolphthalein and 0.2 gm. powdered calcium hydroxide. If solution is not alkaline, add concentrated NH_4OH, drop by drop, until a pink color appears. Make to volume, shake and let stand 30 minutes. Filter through ash-free filter paper, and pipette a 30 ml. aliquot into a 100 ml. beaker. Continue as described for foods.

SODIUM

Analyzed material: **Feces**

Average daily dry weight: **20.8**

Subject:

Balance period:

Date of analysis:

	SAMPLE I[*]	SAMPLE II	SAMPLE III
Volume of sample taken (ml.) Dry wt. of sample taken (gm.)	 **20**	(Fresh urine or ash solution)	
Diluted volume (ml.)	**100**		
Volume of filtrate taken for analysis (ml.)	**6**		
Urine volume in aliquot (ml.) Dry weight in aliquot (gm.)	 **1.2**		
Weight of crucible and precipitate (gm.)	**30.7132**		
Weight of crucible (gm.)	**30.3756**		
Weight of precipitate (gm.)	**0.3376**		
Sodium in aliquot (gm.)	**0.005047**	(Weight of precipitate ×0.01495)	
Sodium per ml. urine (gm.) Sodium per gm. dry weight (gm.)	 **0.004206**	(Sodium in aliquot ÷ volume or dry weight in aliquot)	
Sodium per day (gm.)	**0.087**	(Na per ml. urine or per gm. dry wt. × avg. daily volume or dry wt.)	

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[*] Illustrative values were calculated by machine. Some imply greater accuracy than the procedure warrants (See section on Treatment of Data).

PHOSPHORUS

Phosphorus is determined by Mackay and Butler's modification of the method of Mathison as presented by Peters and Van Slyke.*

Procedure

Urine: Acidify 60 ml. or more of urine with a few drops of concentrated HCl to dissolve any precipitated phosphates, and filter. Transfer 50 ml. of the filtrate (containing 10–100 mg. phosphorus) to a 400 ml. beaker and add 10 ml. of Fiske's magnesia mixture,† followed by 20 ml. of concentrated NH_4OH; stir the solution for one minute, and allow to stand for at least two hours, or over night. Filter the crystalline precipitate into a weighed porous porcelain crucible (Gooch) and wash with 100 ml. or more of dilute NH_4OH (1:5). Ignite at bright red heat (about 1000° C.) in a muffle furnace for one-half hour, cool and weigh the magnesium pyrophosphate ($Mg_2P_2O_7$). In our experience the colorimetric method produces results which may run from 10 to 20 per cent lower than those from the gravimetric method.

Food and feces: Measure an aliquot of ash solution (containing 10 to 100 mg. phosphorus) into a 400 ml. beaker, dilute to 50 ml. and proceed as with urine.

PHOSPHORUS

Analyzed material: **Feces** Balance period: Subject:

Average daily volume (ml.):

Average daily dry weight (gm.): **20.8** Date of analysis

Volume (ml.) / Dry weight (gm.) of sample taken	**10**		
Ash diluted to (ml.)	**100**		
Aliquot taken (ml.)	**20**		
Volume (ml.) / Dry weight (gm.) of sample in aliquot	**2**		
Weight of dish + $Mg_2P_2O_7$ (gm.)	**18.2600**		
Weight of dish (gm.)	**18.0933**		
Weight of $Mg_2P_2O_7$ in aliquot (gm.)	**0.1667**		
Phosphorus in aliquot (gm.)	**0.04641**	($Mg_2P_2O_7$ in aliquot ×0.2784)	
Phosphorus per ml. urine (gm.) / gm. dry weight (gm.)	**0.02322**	(Phosphorus in aliquot ÷ volume or dry weight in aliquot)	
Phosphorus per day (gm.)	**0.483**	(P per ml. urine or per gm. dry wt. ×avg. daily urine vol or dry wt.)	

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* Peters, John P., and Donald D. Van Slyke. Quantitative Clinical Chemistry, Volume II, Methods, p. 859. The Williams and Wilkins Co., Baltimore, 1932. (cf. Ref. 263)

† Fiske's magnesium citrate mixture: Dissolve 265 gm. of citric acid in 350 ml. of hot water. Add, with stirring, 13 gm. of magnesium oxide, free from carbonate. Cool the solution and add 330 ml. of concentrated ammonium hydroxide. Cool and dilute to one liter.

CHLORINE

Chlorine in urine is determined by the Volhard-Arnold method given by Hawk and Bergeim.* The determination in foods and feces is based upon the method of Garelli.†

Procedure

Urine: Place 10 ml. of urine (containing 5 to 10 mg. chlorine) in a 100 ml. volumetric flask; add 20 to 30 drops of concentrated HNO_3 (sp. gr. 1.2) and 2 ml. of a cold, saturated solution of ferric alum. Run in slowly 10 ml. of standard silver nitrate solution,‡ shaking continually. Allow the flask to stand 10 minutes, then fill to the 100 ml. mark with water and mix thoroughly. Filter through a dry filter and pipette a 50 ml. aliquot of the filtrate into another flask. Back titrate the excess silver nitrate in the aliquot with standard ammonium thiocyanate solution.§ The first permanent tinge of red-brown indicates the end-point.

Food and feces: Burn 2 gm. of food or 1 gm. of feces (equivalent to 5 to 10 mg. of chlorine) in the oxygen bomb (see energy procedure, p. 241). Cool the bomb with tap water for 30 minutes to insure complete condensation of the gases, and after cooling, release the pressure slowly. Rinse the bomb and illium capsule thoroughly with hot distilled water, and collect in a 400 ml. beaker. Acidify this solution with concentrated HNO_3 (20 to 30 drops); filter into a volumetric flask, and carry out the procedure described for urine.

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* Hawk, Philip B., and Olaf Bergeim. Practical Physiological Chemistry, 10th Ed., p. 879. P. Blakiston's Son and Co., Inc., Philadelphia, 1931. (cf. Ref. 128)

† Garelli, F. The determination of sulfur, chloride, and bromine in organic products by means of the calorimetric bomb. *Chimie et industrie,* Spec. No. 166, March, 1932. (cf. Ref. 110)

‡ Standard silver nitrate solution: Dissolve 29.061 gm. silver nitrate in one liter of water (1 ml. is equivalent to 6 mg. chlorine).

§ Standard ammonium thiocyanate solution: Solution is made so that 1 ml. of the solution is equivalent to 1 ml. of the standard silver nitrate solution used. Dissolve 13 gm. of ammonium thiocyanate (NH_4SCN) in a little less than a liter of water. In a small flask, place 20 ml. of the standard silver nitrate solution, 5 ml. ferric alum solution and 4 ml. nitric acid (sp. gr. 1.2); add water to make the total volume 100 ml. and thoroughly mix contents of the flask. Run in ammonium thiocyanate solution from a burette until a permanent red-brown tinge is produced, this end-reaction indicating that the last trace of silver nitrate has been precipitated. Take the burette reading and calculate the volume to which the thiocyanate must be diluted to make the two solutions exactly equivalent. Dilute and restandardize.

CHLORINE

Analyzed material: **Urine**

Subject:

Average daily volume (ml.): **1040**
Average daily dry wt. (gm.):

Balance period:

Date of analysis:

	SAMPLE I	SAMPLE II	SAMPLE III
Volume (ml.) / Dry wt. (gm.) of sample taken	**10**		
Volume $AgNO_3$ added (ml.)	**10**		
Volume NH_4CNS used in titration (ml.)	**1.58**		
Volume $AgNO_3$ equivalent to Cl in aliquot (ml.)	**6.84**	($AgNO_3$ added − twice NH_4CNS used in titration)	
Chlorine in sample (mg.)	**41.04**	(Volume of $AgNO_3$ equivalent to Cl in aliquot ×6)	
Chlorine per ml. urine / gm. dry wt. (mg.)	**4.104**	(Cl in aliquot ÷ volume urine or dry wt. in aliquot)	
Chlorine per day (mg.)	**4268**	(Cl per ml. urine or per gm. dry wt. × avg. daily vol. or dry wt.)	

TOTAL SULFUR

Total sulfur is determined in foods and feces upon the washings obtained from the oxygen bomb after combustion for energy determination (see p. 241). Total sulfur in dried urine may also be determined in this way although generally the method outlined under "Sulfur Partition in Urine" has been found more convenient and is carried out with fresh urine samples.

When foods and excreta are burned in the oxygen bomb all of the sulfur is oxidized to SO_3, which dissolves in the water in the bomb to form sulfuric acid. The sulfuric acid may then be determined by precipitation as $BaSO_4$.

Procedure

Filter the acidified bomb washings from the energy determination to remove any particles of ash. Add 10 ml. of 10 per cent barium chloride solution to precipitate barium sulfate. Allow the precipitate to stand several hours, then filter into a weighed, porous porcelain crucible and wash with distilled water until al chlorides have been removed. Ignite the precipitate at 700°C. cool and weigh the barium sulfate.

TOTAL SULFUR

Analyzed material: **Feces**

Subject:

Average daily volume (ml.):

Average daily dry weight (gm.): **23.43**

Balance period:

Date of analysis:

	SAMPLE I*	SAMPLE II	SAMPLE III
Volume of sample (ml.) Dry weight of sample (gm.)	**1.0004**		
Weight of dish + $BaSO_4$ (gm.)	**22.8814**		
Weight of dish (gm.)	**22.8407**		
Weight of $BaSO_4$† (gm.)	**0.0407**		
Sulfur in sample (gm.)	**0.005592**	($BaSO_4$ in sample ×0.1374)	
Sulfur per ml. urine (mg.) Sulfur per gm. dry weight (gm.)	**0.005590**	(Sulfur in sample ÷ dry weight of sample)	
Sulfur per day (gm.)	**0.131**	(Sulfur per gram sample × average daily dry weight)	

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* Illustrative values were calculated by machine. Some imply greater accuracy than the procedure warrants (See section on Treatment of Data).

† $BaSO_4$ is slightly soluble in water at room temperature. When large volumes of water have been used and small $BaSO_4$ samples are weighed, the solubility factor should be taken into account.

SULFUR PARTITION IN URINE

Total sulfur in urine may be determined by combustion of dried urine samples in the oxygen bomb (p. 269). It is more convenient to determine urinary total sulfur by an adaptation of the methods of Folin* and Benedict.† The method is not applicable to foods and feces.

Procedure

Total sulfur: To 25 ml. of urine in a porcelain evaporating dish (flat bottom, 8 cm. in diameter) add 10 ml. of Benedict's total sulfur reagent.‡ Evaporate to dryness on a steam bath. Heat on a hot plate until the solid material is blackened, exercising care not to heat too fast and cause the material to burn explosively. Ignite in a muffle furnace at 600 to 700° C. for 30 minutes. Cool and dissolve the residue in 10 to 20 ml. of dilute HCl (1:4). Filter solution into a 400 ml. beaker to remove particles of porcelain which may have chipped from the dish during the ignition, and wash filter with 150 ml. of hot distilled water. Precipitate barium sulfate by dropwise addition of 10 ml., 10 per cent barium chloride solution and, after allowing the precipitate to stand over night, filter through a porcelain Akatos A-1 (or Gooch) crucible which previously has been ignited and weighed. Wash the precipitate with distilled water until wash solution is free of chloride, dry the crucible, ignite at 750° C. and weigh.

Inorganic sulfur: Measure accurately into a 400 ml. beaker, 100 ml. of urine. Add 30 ml. of dilute HCl (1:4) and mix. Add 20 ml., 10 per cent barium chloride solution, drop by drop, without stirring. Cover with a watch glass to prevent evaporation and allow to stand two hours. Mix the solution and filter, wash, ignite and weigh as for total sulfur. Retain the *original* filtrate, undiluted with wash water, for ethereal sulfur determination.

Ethereal sulfur: Pipette 100 ml. of the filtrate from the inorganic sulfur determination (represents 66.6 ml. of undiluted urine) into a 400 ml. beaker. Cover with a watch glass and boil on hot plate, hydrolyzing the ethereal sulfates to free sulfuric acid. Barium sulfate is precipitated by the barium chloride present in the solution. After 30 minutes boiling cool the solution and, after allowing the precipitate to stand several hours, filter, wash, ignite and weigh the precipitate as for total sulfur.

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* Folin, Otto. On sulphate and sulphur determinations. *J. Biol. Chem.*, 1: 131, 1905. (cf. Ref. 105)

† Benedict, Stanley R. The estimation of total sulphur in urine. *J. Biol. Chem.*, 6: 363, 1909. (cf. Ref. 13)

‡ Benedict's total sulfur reagent: Dissolve 200 gm. copper nitrate and 50 gm. potassium chlorate in distilled water and dilute to one liter.

IRON

Iron is determined by the method presented by Hummel and Willard.[*]

Procedure

Urine: Dry 500 ml. of urine in a porcelain evaporating dish on steam bath, or in steam oven, and ash over night in an electric muffle furnace at 500° C. Dissolve the inorganic residue in dilute HCl (1:2) and filter through ash-free paper into a 50 ml. volumetric flask.

Transfer one aliquot[†] (representing 0.1 to 0.6 mg. iron) of the ash solution to a 25 ml. volumetric flask, and another aliquot (same size), to a test tube. To the aliquot in the test tube add five drops of bromphenol blue indicator solution[‡] and from a burette add 2 *M* sodium acetate until the solution reaches *pH* 3.5, the turning point of the indicator. Add this determined amount of the sodium acetate solution to the corresponding aliquot in the 25 ml. volumetric flask, followed by 1 ml. of one per cent hydroquinone solution[§] (in acetate buffer, *pH* 4.5) and 1 ml. of 0.5 per cent o-phenanthroline solution.[+] Make solution to volume with water, mix and allow to stand at least one hour for the maximum color to develop. Read in photelometer, using a solution of the reagents as a blank. The concentration of iron is read from a standard curve derived by plotting results obtained by the same method with varying concentrations of standard iron solutions.

Foods, feces: Ash 20 gm. dried food, or 5 gm. dried feces, in the manner given for urine and follow same procedure. It is always desirable to re-ash the filter paper containing the insoluble residue from food or feces. Dissolve the ash in dilute HCl (1:2) and add to the original filtrate.

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[*] Hummel, Frances Cope, and H. H. Willard. Determination of iron in biological materials. The use of o-phenanthroline. *Ind. Eng. Chem.*, Analyt. Ed., 10: 13, 1938. (cf. Ref. 157)

[†] It is frequently necessary to take a 20 ml. aliquot, due to a very small amount of iron present. In such cases 5 *M* sodium acetate is used to adjust the *pH* to 3.5 and a few crystals of o-phenanthroline and 100 mg. of hydroquinone are added; the solutions are read in the photelometer as before; often necessary in the analysis of urine.

[‡] Bromphenol blue indicator solution, 0.04 per cent. La Motte Chemical Products Co., Baltimore, Md.

[§] Hydroquinone solution, one per cent. Dissolve 1 gm. of hydroquinone in a sodium citrate acetic acid buffer solution with a *pH* of 4.5.[‖] Keep the solution in the refrigerator and discard as soon as any color develops.

[‖] Clark, W. M. The determination of hydrogen ions. The Williams and Wilkins Co., 3rd ed., Baltimore, 1928. (cf. Ref. 49)

[+] Orthophenanthroline solution, 0.5 per cent. Dissolve 0.5 gm. of orthophenanthroline monohydrate (G. Frederick Smith Co., Columbus, Ohio) in 100 ml. of distilled water, and warm to effect solution.

IRON

Analyzed material: **Feces**

Subject:

Average daily volume (ml.):

Average daily dry weight (gm.): **20.8**

Balance period:

Date of analysis:

Sample prepared by (method):

	SAMPLE I	SAMPLE II	SAMPLE III
Volume of sample taken (ml.)			
Dry weight of sample taken (gm.)	**10**		
Dry weight in aliquot (gm.)	**1**		
Photelometer reading	**14.75**		
Iron in aliquot (from curve) (mg.)	**0.417**		
Iron per ml. urine (mg.)			
Iron per gram dry weight (mg.)	**0.417**		
Iron per day (mg.)	**8.674**		

MANGANESE

Manganese in food and feces may be determined by a modification of the colorimetric method of Willard and Greathouse.[*] The method is also applicable to urine, but the small amounts of manganese present in the samples from the experimental subjects of our studies made colorimetric comparison very difficult and for this reason manganese in urine was determined polarographically (p. 363).

Procedure

Food, feces: Evaporate an aliquot of ash solution of food or feces (containing at least 0.15 mg. of manganese) to dryness in a beaker, with care to avoid splattering. Heat the residue until all excess HCl is driven off. When cool, add 5 ml. of concentrated HNO_3 and 3 to 5 ml. of water; warm to dissolve the residue. Cool the solution, add 3 to 4 drops of concentrated H_2SO_4 and evaporate to dryness or until SO_3 fumes are given off. Considerable care must be exercised to avoid splattering during the evaporation. Add 20 to 25 ml. of 15 per cent HNO_3 and warm the beaker to dissolve the residue; add a few more milliliters of water if necessary for solution. Remove any precipitate of $CaSO_4$ by filtering through a small filter paper and wash with a few milliliters of water.

To the filtrate, in a 50 ml. centrifuge tube, add 0.2 to 0.3 gm. of potassium periodate and 0.5 ml. of syrupy phosphoric acid. Since iron salts give a yellow color to the solution, the phosphoric acid is added to form a colorless complex with the ferric iron. Heat the tubes in a boiling water bath 15 to 20 minutes, or until full development of the permanganate color.[†] Carry a standard solution of manganese throughout the procedure, as a standard for color comparison. Cool the tubes, transfer contents to a 25 ml. volumetric flask, dilute to volume, and read in colorimeter.[‡]

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[*] Willard, H. H., and L. H. Greathouse. The colorimetric determination of manganese by oxidation with periodate. *J. Am. Chem. Soc.*, 39: 2366, 1917 (cf. Ref. 380).

[†] When KIO_4 is used as an oxidizing agent, the permanganate color is not affected by variation of the acid concentration within wide limits, by prolonged boiling, or by long standing (several months).[380]

[‡] In determining manganese in materials such as milk and potatoes, which contain extremely small amounts (0.2 μg. and 4.3 μg. per gm. dry weight, respectively) the procedure must be slightly varied. Remove the chlorides by evaporation with nitric acid, as described above, from an ashed sample representing at least 15 gm. of dried milk, or 1.5 gm. of dried potatoes. Larger samples should be used if available, since the manganese in these samples provides the minimum $KMnO_4$ color which can be read in the special Nessler tubes used. Dissolve the residue in 15 ml. of 15 per cent HNO_3 and remove the slight precipitate of $CaSO_4$ by filtration. To the filtrate, in titer tubes, add 0.2 gm. KIO_4 and heat the tubes 40 to 50 minutes in a boiling water bath. After the color has developed, cool the solution, transfer to a 10 ml. volumetric flask, dilute to volume, and compare the color against the standard, similarly treated, in special Nessler tubes of 10 ml. capacity and approximately 12 cm. in length, graduated at 0.1 cm. intervals.[276]

TOTAL ORGANIC ACID IN URINE

In the titration of total organic acid in urine the method of Van Slyke and Palmer is used as presented in Peters and Van Slyke.*

Procedure

Place approximately 100 ml. of urine in a 250 ml. beaker. If urine is not acid to litmus, add a few drops of 10 per cent HCl and shake vigorously until most of the liberated carbon dioxide has been driven off. Add 2 gm. powdered calcium hydroxide, mix thoroughly and let stand about 15 minutes, stirring occasionally. Filter through a dry, folded filter. As a control, run a blank with each series of determinations, substituting water for urine.

Measure 25 ml. of the filtrate into a pyrex test tube, 125 or 150 ml. capacity, 25 or 30 mm. diameter, with a calibration mark at 60 ml. Add 0.5 ml. of one per cent phenolphthalein solution in 50 per cent alcohol and titrate with 0.2 N HCl, from a burette, until the pink color just disappears from the solution (pH 8.0). Prepare a reference tube by placing 0.6 ml. 0.2 N HCl and 5 ml. 0.02 per cent tropeolin OO solution† in another calibrated test tube and add distilled water to make 60 ml.

Add gradually to the unknown, 5 ml. 0.02 per cent tropeolin OO, while constantly shaking the tube to insure mixture of its contents. (If the shaking is omitted some of the dye may be precipitated.) Titrate the solution with 0.2 N HCl until it attains a red color that matches the color of the solution in the reference tube. As the end point is approached, add water to the unknown to bring its volume to 60 ml.

Comparison of the color of the titrated solution with the color of the solution in the reference tube is conveniently made throughout the titration by holding the two tubes side by side between the thumb and forefinger, with the unknown nearer the tips of the fingers where it can easily be shaken as the acid is added. All tubes containing unknowns should be allowed to stand 15 minutes after they are titrated, compared with their respective reference solutions and, if any fading has occurred after the acid end-point of *pH* 2.7 is reached, the titration should be repeated, using bromphenol blue,* instead of tropeolin OO as indicator.

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* Peters, John P., and Donald D. Van Slyke. Quantitative Clinical Chemistry, Volume II, Methods, p. 647. The Williams and Wilkins Co., Baltimore, 1932. (cf. Ref. 263)

† Tropeolin OO solution, 0.02 per cent in water.

TOTAL ORGANIC ACID IN URINE

Total volume for day (ml.): **1000**

Subject:

Balance period:

Date of analysis:

	SAMPLE I	SAMPLE II	SAMPLE III
Volume taken for analysis (ml.)	**25**		
HCl for titration (ml.)	**5.46**		
HCl for blank titration (ml.)	**0.64**		
Corrected titration (ml.)	**4.82**		
Normality of HCl	**0.2046**		
Normal HCl used in titration (ml.) (Each ml. equals 1 meq.)	**0.9862**	(Corrected titration ×normality of HCl)	
Organic acid per ml. of sample (meq.)	**0.0394**	(Normal HCl used in titration ÷volume of sample)	
Organic acid per day (meq.)	**39.4**	(Organic acid per ml. sample ×urine volume for day)	
Creatinine nitrogen per day (mg.)*	**331**		
Sixty per cent of creatine nitrogen per day (mg.)*	**6.6**		
Total creatinine correction for day (meq.)	**8.0**	(Sum of creatinine and 60 per cent of creatine nitrogen ÷42)	
Total organic acid per day (meq.)	**31.4**	(Organic acid −creatinine correction)	

* From record of creatinine and creatine determination.

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* Bromphenol blue, 0.04 per cent solution. To 100 mg. bromphenol blue add 14.9 ml. of 0.01 *N* NaOH and dilute to 250 ml. with water.

TITRABLE ACIDITY OF URINE

Total acidity of urine is determined by the method of Henderson and Palmer as given by Peters and Van Slyke.*

Procedure

Place 10 ml. of phosphate solution† in a 500 ml. flask, dilute to 250 ml. with water and add 2 ml. phenol red solution‡ or 0.2 ml. of two per cent neutral red solution. In another 500 ml. flask, dilute 10 ml. of urine to 250 ml. with water: add indicator and titrate with standard 0.1 *N* NaOH until a color which matches the phosphate standard is attained.

TITRABLE ACIDITY OF URINE

Total volume per day (ml.): **1000**

Subject:

pH:

Balance period:

Date of analysis:

	SAMPLE I	SAMPLE II	SAMPLE III
Volume of sample taken (ml.)	**10**		
Volume of NaOH used (ml.)	**1.54**		
Normality of NaOH	**0.1176**		
Volume of 0.1 *N* NaOH used (ml.)	**1.811**	(Volume of NaOH used ×normality of NaOH ×10)	
Acidity per ml. sample (ml. 0.1 *N* NaOH)	**0.1811**	(Volume 0.1 *N* NaCl used ÷ volume of sample)	
Total acidity for day (ml. 0.1 *N* HCl)	**181**	(Acidity per ml. sample ×urine volume for day	

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* Peters, John P., and Donald D. Van Slyke. Quantitative Clinical Chemistry, Volume II, Methods, p. 827. The Williams and Wilkins Co., Baltimore, 1932. (cf. Ref. 263)

† Phosphate solution, *pH* 7.4. Eighty and eight-tenths (80.8) milliliters of 0.067 *M* disodium phosphate (9.47 gm. anhydrous Na_2HPO_4 per liter) and 19.2 miillilters of 0.067 *M* potassium acid phosphate (9.08 gm. KH_2PO_4 per liter).

‡ Phenol red: Add 5.7 ml. 0.05 *N* NaOH to 0.1 gm. phenol red and dilute to 100 ml.

NITROGEN

Nitrogen is determined by the macro-Kjeldahl procedure of Scales and Harrison.*

Procedure

Measure an aliquot (containing 20 to 50 mg. of nitrogen) of urine or of sulfuric acid digest preparation of food or feces (see page 240) into a 500 ml. Kjeldahl flask and digest with 30 ml. concentrated H_2SO_4 and 5 to 10 gm. digestion mixture.† Digestion, which is obtained by boiling the sample, should be continued for one-half hour (urine) to 1 hour or longer (feces or food) after sample becomes clear. Dilute to about 250 ml. with distilled water, cool, add several small pieces of mossy zinc and enough 60 per cent NaOH (70 to 100 ml.) to neutralize the acid, being careful to form a layer with the alkali. Attach the flask to distilling apparatus, mix contents by shaking without disturbing connection to distilling apparatus, and distill, collecting the distillate in 75 ml. of a saturated solution of boric acid‡ in a 500 ml., wide-mouth Erlenmeyer flask. After the distillation is completed (approximately 150 ml.) titrate the ammonia directly with standard HCl,§ using methyl red-methylene blue‖ as indicator.

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* Scales, F. M., and A. P. Harrison. The boric acid modification of the Kjeldahl method. *Ind. Eng. Chem.*, 12: 350, 1920. (cf. Ref. 288)

† Digestion mixture: One hundred grams $Na_2SO_4 + 3$ gm. $CuSO_4$ (hydrated salts may be used).

‡ Saturated solution contains approximately 4 per cent boric acid. The boric acid solution may be measured with a graduate.

§ Simultaneously with determination of nitrogen in the sample, carry through the procedure a blank, using a 5 cm. disc of filter paper or one gram of sugar instead of the sample. In the titration, the sample is titrated to match the color of the blank.

‖ Methyl red-methylene blue: Dissolve 500 mg. methylene blue in 100 ml. 95 per cent ethanol. Dissolve 500 mg. methyl red in 100 ml. 95 per cent ethanol. Add one part methylene blue solution to 15 parts of methyl red solution. One part of the mixed solution is diluted with nine parts of 50 per cent ethanol to make the indicator solution.

NITROGEN

Analyzed material: **Feces**

Subject:

Average daily wet wt. (gm.):

Average daily volume (ml.): **349**

Balance period:

Date of analysis:

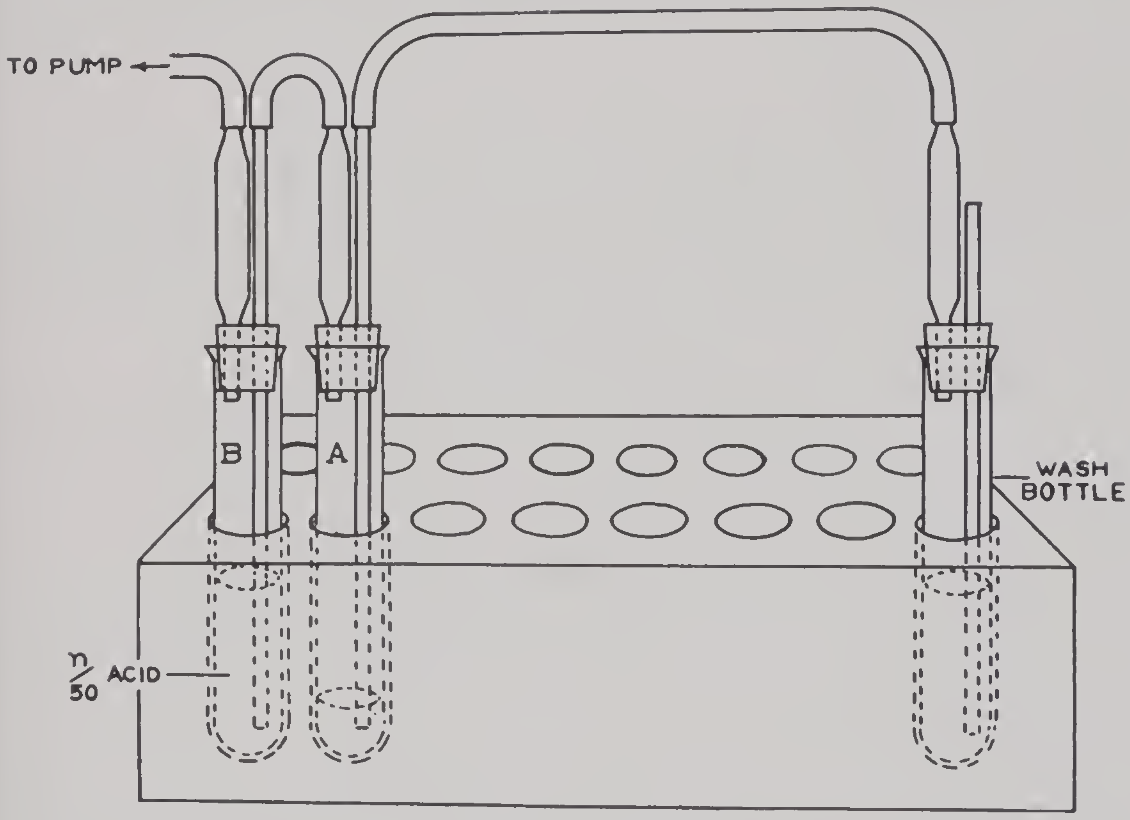
	SAMPLE I*	SAMPLE II	SAMPLE III
Wet wt. of sample (gm.) Volume of sample (ml.)	**256**		
Diluted to (ml.)	**500**		
Wet weight per ml. sample (gm.)	**0.512**	(Wet weight ÷ volume)	
Volume of aliquot taken (ml.)	**15**		
Wet weight in aliquot (mg.)	**7.68**	(Volume of aliquote × wet weight per ml.)	
Initial reading	**18.00**		
Final reading	**35.90**		
HCl used (ml.)	**17.90**		
Normality of HCl	**0.1175**		
Normal HCl equivalent (ml.)	**2.10325**	(Normal HCl used × normality of HCl)	
Nitrogen in aliquot (mg.)	**29.4455**	(HCl equivalent × 14)	
Nitrogen per ml. urine Nitrogen per gm. wet wt. feces (mg.)	**3.8340**	(Nitrogen in aliquot ÷ volume of urine or wet wt. in aliquot)	
Nitrogen per day (mg.)	**1338**	(Nitrogen per ml. urine or gm. wet wt. × avg. daily volume or wet wt.)	

* Illustrative values were calculated by machine. Some imply greater accuracy than the procedure warrants (See section on Treatment of Data).

NITROGEN PARTITION OF URINE

UREA AND AMMONIA

The standard procedure for the analysis of urine for urea and ammonia was developed by Van Slyke and Cullen* and provides for the determination of the sum of urea and ammonia nitrogen, in a sample treated with urease, which converts urea into ammonium carbonate. Ammonia nitrogen alone is determined in another sample and the urea nitrogen content of the urine is then calculated by difference.



The apparatus consists of pyrex test tubes, 250×20 mm., arranged in pairs and provided with aspirator tubes and connected so that air may be drawn through them in series. An extra tube containing dilute H_2SO_4 is inserted before the series to remove traces of ammonia which might be present in the air.

Procedure

Urea plus ammonia: Dilute 5 ml. of urine to 50 ml. with water. Measure 5 ml. of the diluted urine into one of the test tubes, add a drop of caprylic alcohol, 5 ml. phosphate buffer† and one tablet of urease preparation,* crushed. Stopper the test tube with the aspirator and shake; after allowing to stand 30 minutes at room temperature, add 7 ml. of saturated sodium carbonate solution and connect the sample tube with a similar tube containing 25 ml. of 0.02 *N* HCl to which a drop of caprylic alcohol and two to five drops of indicator† have been added (See p. 279). Aspirate with a current of air until all the ammonia has been carried from the sample into the standard acid. The time needed for

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* Peters, John P., and Donald D. Van Slyke. Quantitative Clinical Chemistry, Volume II, Methods, p. 545. The Williams and Wilkins Co., Baltimore, 1932. (cf. Ref. 263)

† Phosphate buffer. Dissolve 6 gm. of potassium di-hydrogen phosphate (KH_2PO_4) and 2 gm. of anhydrous di-sodium hydrogen phosphate (Na_2HPO_4 or 5 gm. $Na_2HPO_4 \cdot 12H_2O$) in one liter of water.

AMMONIA AND UREA PLUS AMMONIA NITROGEN IN URINE

Subject:

Volume for day (ml.): **1000**

Balance period:

Date of analysis:

Date of collection:

	SAMPLE I*		SAMPLE II	
	NH_3	Urea + NH_3	NH_3	Urea + NH_3
Volume of sample (ml.)	5	5		
Diluted volume (ml.)	5	50		
Volume of aliquot (ml.)	5	0.5		
NaOH used in titration (ml.)	22.51	9.13		
Normality of NaOH	0.01774	0.01774		
Normal NaOH used (ml.)	0.3993	0.1620		
Volume of HCl added (ml.)	25	25		
Normality of HCl	0.0200	0.0200		
Volume of *N* HCl in sample (ml.)	0.50	0.50		
Volume *N* HCl neutralized (ml.)	0.1007	0.3380	(Normal HCl in sample —normal NaOH used)	
Nitrogen in aliquot (mg.)	1.410	4.732	(Volume of HCl neutralized by $NH_3 \times 14$)	
Nitrogen per ml. of urine (mg.)	0.282	9.464	(Nitrogen in aliquot ÷ urine volume in aliquot)	
Nitrogen per day (mg.)	282	9464	(Nitrogen per ml. urine × daily urine volume)	

* Illustrative values were calculated by machine. Some imply greater accuracy than the procedure warrants (See section on Treatment of Data).

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* Urease: Urease tablets (100 mg.) supplied by Hynson, Westcott and Dunning, Baltimore, are satisfactory. Other satisfactory enzyme preparations are described by Peters and Van Slyke[263] and Hawk and Bergeim[128].

† Methyl red-methylene blue indicator: Dissolve 1.25 gm. methyl red and 0.825 gm. methylene blue in 1 liter of 90 per cent alcohol.

aspiration is dependent upon the speed of the air current used. With a reasonably good water pump, 16 pairs of tubes may be aspirated in series in 30 minutes. After the aspiration is complete, disconnect the standard acid tubes before the suction is turned off, *starting at the end farthest from the water pump.* Titrate the excess acid with 0.02 *N* NaOH.

Ammonia: Carry out the ammonia determination in a manner identical with that described for *Urea plus ammonia,* but use a 10 ml. sample of *undiluted* urine and do not add urease. Absorb the ammonia in 25 ml. of standard 0.02 *N* HCl and, after completion of the aspiration, back titrate the excess acid with standard 0.02 *N* NaOH.

URIC ACID

All the colorimetric methods for the determination of uric acid depend upon the formation of a blue color in the presence of tungstic acid or a derivative of it. The method applied routinely to urine was developed by Benedict and Franke.*

URIC ACID IN URINE

Urine volume per day (ml.): **1000**

Subject:

Balance period:

Date of determinations:

	SAMPLE I	SAMPLE II	SAMPLE III
Volume of sample taken (ml.)	5		
Diluted volume (ml.)	50		
Aliquot taken (ml.)	5		
Volume of sample in aliquot (ml.)	0.5		
Setting of standard (mm.)	20		
Uric acid in standard (mg. 50 ml.)	0.20		
Reading of unknown (mm.)	17.4		
Uric acid in aliquot (mg.)	0.2299	(Setting of standard × uric acid in standard ÷ reading for unknown)	
Uric acid per ml. urine (mg.)	0.4598		(Uric acid in aliquot ×2)
Uric acid per day (mg.)	459.8	(Uric acid per ml. urine × daily volume)	
Uric acid nitrogen per day (mg.)	153		(Uric acid per day ×0.333)

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* Benedict, Stanley R., and Elizabeth Franke. A method for the direct determination of uric acid in urine. *J. Biol. Chem.,* 52: 387, 1922. (cf. Ref. 15)

Procedure

Dilute 5 ml. of urine to 50 ml. with distilled water. (This is the same dilution employed for the urea determination.) Measure 5 ml. of this diluted urine into a 50 ml. volumetric flask. Add 5 ml. of water and 5 ml. of five per cent sodium cyanide solution* (cyanide solutions should always be measured from a burette), followed by 1 ml. of the arsenophosphotungstic acid reagent.† Mix the solution and allow to stand five minutes (if allowed to stand much longer, turbidity develops); then dilute to the mark and mix. The color developed in the sample is compared in the colorimeter with a standard, simultaneously prepared with 10 ml. of standard uric acid solution‡ (0.02 mg. per ml.). To avoid the development of turbidity no more samples should be handled at one time than can be read within 15 minutes after development of color. In comparing the solutions in the colorimeter, the standard should be set 20 mm. in depth.

CREATININE AND CREATINE

Creatinine is determined by the Jaffe color reaction. Creatinine in the presence of picric acid forms creatinine picrate which yields a red tautomer in the presence of alkali. Folin determined the conditions for using the color reaction quantitatively.§

Creatine is converted to creatinine by boiling in the presence of acid. Urine treated this way may then be used for the colorimetric determination of preformed creatinine plus the creatinine arising from creatine. Conversion of creatine to creatinine by procedures such as Folin's open flask and autoclave methods are not always dependable. Frequently incomplete conversion results. In our hands the method developed by Benedict has been more uniformly satisfactory.* Some conversion of creatinine to creatine may occur during storage of urine samples,† therefore, creatinine and creatine determinations are only valid when done within a short time after the 24-hour urine sample is collected. Our practice has been to do these determinations the day samples are collected.

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* Sodium cyanide: Five per cent solution, containing 2 ml. of concentrated NH_4OH. Stable for about six weeks.

† Arsenophosphotungstic acid reagent: Place 100 gm. of sodium tungstate (Baker's) in a one liter pyrex flask and dissolve in 600 ml. of water. Add 50 gm. of arsenic pentoxide (As_2O_5); then 25 ml. of 85 per cent phosphoric acid and 20 ml. of concentrated HCl. Boil the mixture for 20 minutes and dilute to I liter.

‡ Folin's standard uric acid solution: To 0.6 gm. of lithium carbonate in a 250 ml. flask, add 150 ml. of water and shake until the salt is dissolved. Filter the lithium carbonate solution to remove any precipitate. Place I gm. uric acid in a liter volumetric flask and warm under the hot water tap. Heat the lithium carbonate solution to 60° C. and pour into the liter flask containing the uric acid, washing down any uric acid crystals adherent to the neck. Shake the warm solution five minutes and then cool immediately under running cold water. Add 20 ml. of formaldehyde (50 per cent solution) and half fill the flask with water. Add a few drops of methyl orange and then slowly, with shaking, add 25 ml. *N* H_2SO_4. (The solution should turn pink while 2.5 to 3 ml. of acid remain in the pipette.) Dilute the solution to a liter. Mix, place in a tightly stoppered bottle and keep in a dark place. The stock solution is stable indefinitely. For use, dilute 10 ml., in a 500 ml. volumetric flask, with 350 ml. of water and 25 ml. of HCl (1:10). Add water to mark. The dilute solution should be prepared every three or four days.

§ Folin, Otto, On the determination of creatinine and creatine in urine. *J. Biol. Chem.*, 17: 469, 1914. (cf. Ref. 106)

Procedure

Creatinine: Place 1 to 2 ml. of urine in a 100 ml. volumetric flask. In another, place 1 ml. of a standard solution of creatinine‡ (1 mg.). To each flask, add 20 ml. of saturated picric acid and 1.5 ml. of 10 per cent NaOH (both should be measured accurately in burettes). After mixing, allow the solutions to stand five minutes. Dilute to the 100 ml. mark and read in the colorimeter. Daylight is the best source for comparison. (The photelometer may be substituted for a colorimeter.)

If the colorimeter is used, the standard should be set at the depth of 8 mm., originally recommended by Folin. With the standard at this depth, color changes may be distinguished with greater accuracy than if the standard is set at 20 mm.

Creatinine plus creatine: Measure 1 to 2 ml. of urine into a 125 ml. Erlenmeyer flask and add 10 ml. *N* HCl. Evaporate the contents of the flask to a syrupy consistency on a hot plate and dry on a steam bath. The sample should not be dried on the hot plate as the attendant burning interferes with the determination. To the dried sample, add 20 ml. of saturated picric acid and 1.5 ml. of 10 per cent NaOH and let stand for five minutes. Wash the contents of the flask quantitatively into a 100 ml. volumetric flask and dilute to the mark. Compare in the colorimeter as in the creatinine determination, with a simultaneously prepared standard solution containing 1 mg. of creatinine.

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* Benedict, Stanley R., Studies in creatine and creatinine metabolism. II. The estimation of creatine. *J. Biol. Chem.*, 18: 191, 1914. (cf. Ref. 14)

† Horvath, S. M., and D. B. Dill. The conversion of creatinine into creatine in normal urine. *J. Lab. Clin. Med.*, 26: 1673, 1941. (cf. Ref. 148)

‡ Standard creatinine solution: Dissolve 1.602 gm. of creatinine zinc chloride in 1 liter 0.1 *N* HCl; 1 ml. of this solution contains 1 mg. creatinine.

Evaporation of the urine to dryness with hydrochloric acid to convert creatine to creatinine is based upon Benedict's method; however, we have avoided the use of powdered lead in boiling, suggested by Benedict to prevent the formation of dark colored compounds. This omission is considered advisable in view of the fact that, even when visible particles of powdered lead are filtered out, the lead treatment yields high values for total creatinine. Perhaps this is due to invisible particles of lead or some other reducing substance in solution which converts picric acid to red picramic acid. The slight darkening of the urine on boiling does not interfere appreciably with accurate comparison of the samples in the colorimeter. This fact has been proven in samples of urine containing no creatine. Such creatine free samples yield identical creatinine values before and after boiling with hydrochloric acid and added creatine may be quantitatively recovered.

CREATINE* AND CREATININE IN URINE

Total volume for day: **1000**

Subject:

Balance period:

Date of analysis:

	Preformed creatinine	Creatine + creatinine†	Preformed creatinine	Creatine + creatinine
Volume of sample (ml.)	**2**	**1**		
Diluted volume (ml.)	**100**	**100**		
Reading of standard (mm.)	**8.0**	**8.0**		
Creatinine in standard (mg./100 ml.)	**2**	**1**		
Reading of unknown (mm.)	**9.0**	**8.7**		
Creatinine in sample (mg.)	**1.7778**	**0.9195**	(Reading of standard × creatinine in standard ÷ reading for unknown)	
Creatinine per ml. urine (mg.)	**0.8889**	**0.9195**	(Creatinine in sample ÷ urine volume in sample)	
Creatinine per day (mg.)	**889**	**920**	(Creatinine per ml. urine × daily volume)	
Creatinine nitrogen per day (mg.)	**331**	**342**	(Creatinine per day × 0.372).	

* Creatine nitrogen is calculated as creatine nitrogen + creatinine nitrogen − preformed creatinine nitrogen.

† In terms of creatinine.

MANOMETRIC CARBON DETERMINATION

The method for manometric carbon determination was developed by Van Slyke and Folch.* The procedure for microanalysis is reproduced through the courtesy of the authors and *The Journal of Biological Chemistry*.

The method depends on combustion in a mixture of chromic, iodic, sulfuric, and phosphoric acids. With all types of organic substances thus far tried this mixture gives theoretical yields of carbon dioxide with one to three minutes boiling. The CO_2 is absorbed in alkali solution in the Van Slyke-Neill manometric apparatus and then determined by a development, modified in most details, of the procedure introduced by Backlin† and refined by Van Slyke, Page, and Kirk.‡ For absorption of the CO_2 a solution of NaOH and hydrazine is used, the hydrazine serving to reduce any halogens evolved during the combustion.

The same apparatus serves for *submicroanalyses* with 0.3 to 0.7 mg. of carbon, for *microanalyses* with 2 to 3.5 mg., and for *macroanalyses* with 8 to 15 mg. In the submicroanalyses the mean error is of the order of one part in 200, while in the micro and macro it is of the order of one in 500. The time routinely required for an analysis, after the sample has been weighed or measured, is about 15 minutes.

Apparatus§

The apparatus used for the combustion is shown in Figs. 1 and 2. With changes in details it is the same used by Van Slyke, Page, and Kirk.‡ The combustion tube, *T*, and the connecting tube *Q* are made of Pyrex glass. The core of the cock below *F* must also be of Pyrex glass, or unequal expansion when the apparatus warms in use may cause the cock to stick.

Accessory apparatus is shown in Figs. 3 and 4.

In Fig. 5 is shown the construction of a sheet aluminum scoop and counterpoise that have proved convenient for weighing samples on the microbalance. For handling the scoop one uses small crucible tongs with chamois skin glued to the inside of the jaws.

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* Van Slyke, Donald D., and Jordi Folch. Manometric Carbon Determination. *J. Biol. Chem.*, 136: 509, 1940. (cf. Ref. 356)

† Backlin, E. *Biochem. Z.*, 217: 483, 1930.

‡ Van Slyke, Donald D., Irving H. Page, and Esben Kirk, *J. Biol. Chem.*, 102: 635, 1933.

§ The apparatus can be obtained from Eimer and Amend, and from E. Machlett and Son, New York.

If the scoop were handled with unprotected metal jaws, the aluminum would be worn off at a rate which would rather rapidly diminish its weight.

Alundum (crystalline alumina) pieces to promote smooth boiling of the combustion mixture are cleaned by boiling them in combustion fluid, washing with water, and drying. They are kept protected from dust in a small Petri dish. The pieces, 1 to 2 mm.

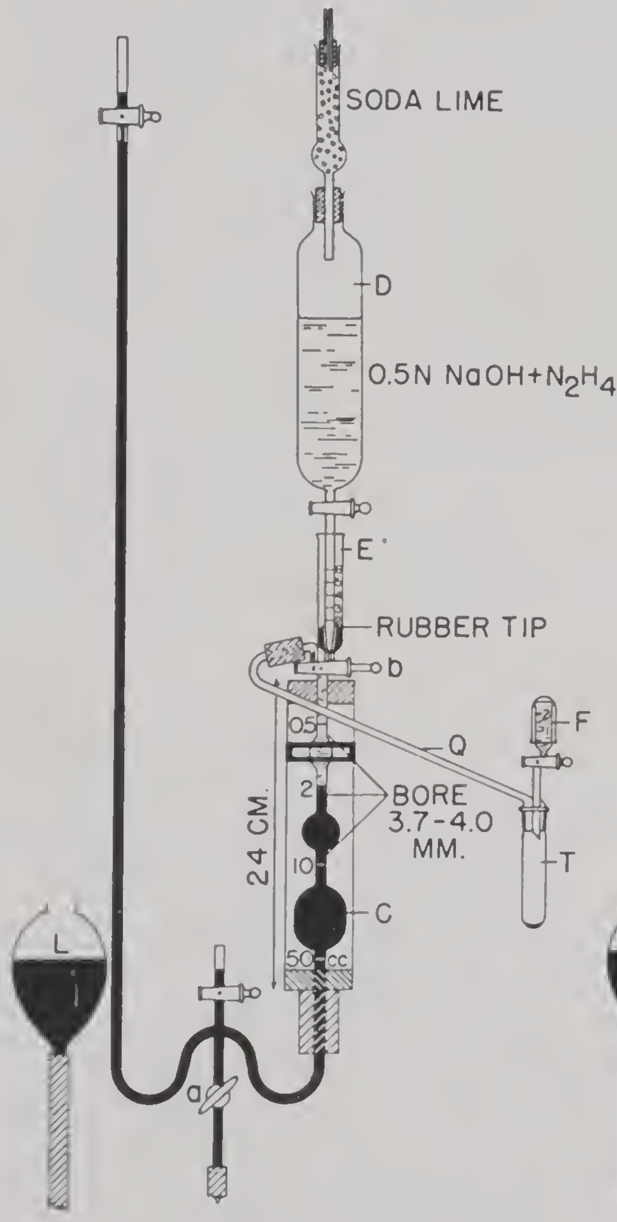

FIG. 1

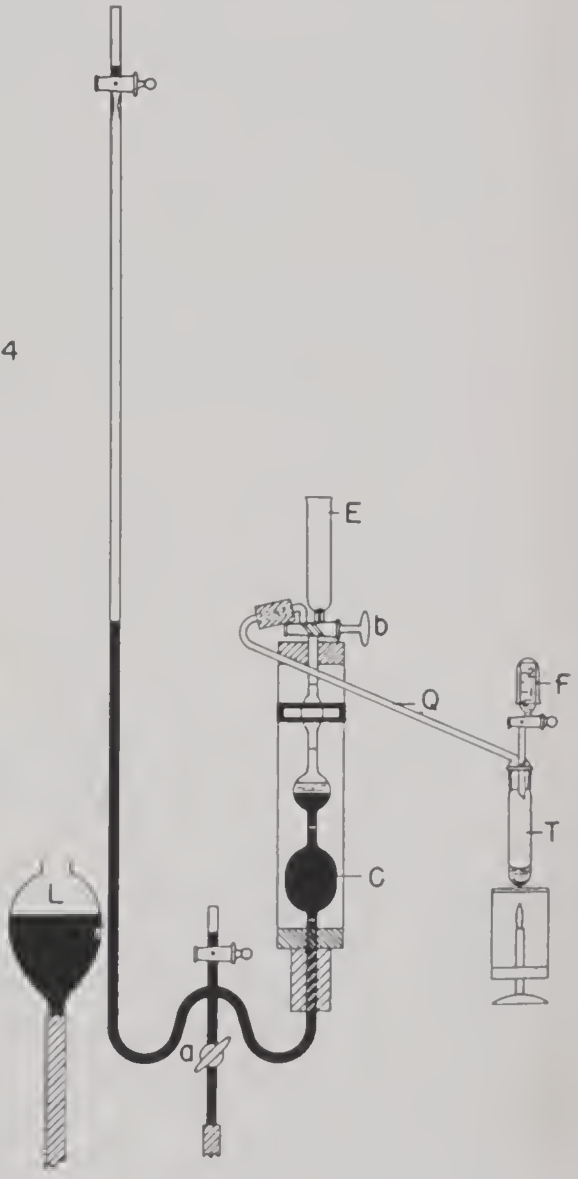

FIG. 2

FIGURE 1. Apparatus after delivery of alkali into chamber, and before the start of the combustion.—After Van Slyke and Folch.[356]

FIGURE 2. Apparatus at start of combustion. The chromic acid solution has been run into the combustion tube and the flame has just been brought under it, but, as shown by the low level of the mercury in the manometer, evolution of CO_2 and O_2 has not yet begun.—After Van Slyke and Folch.[356]

long, are size No. 14 of the Norton Company. Presumably sand of similar particle size would do as well.

Reagents: Chromic acid combustion fluid. In a one liter Pyrex

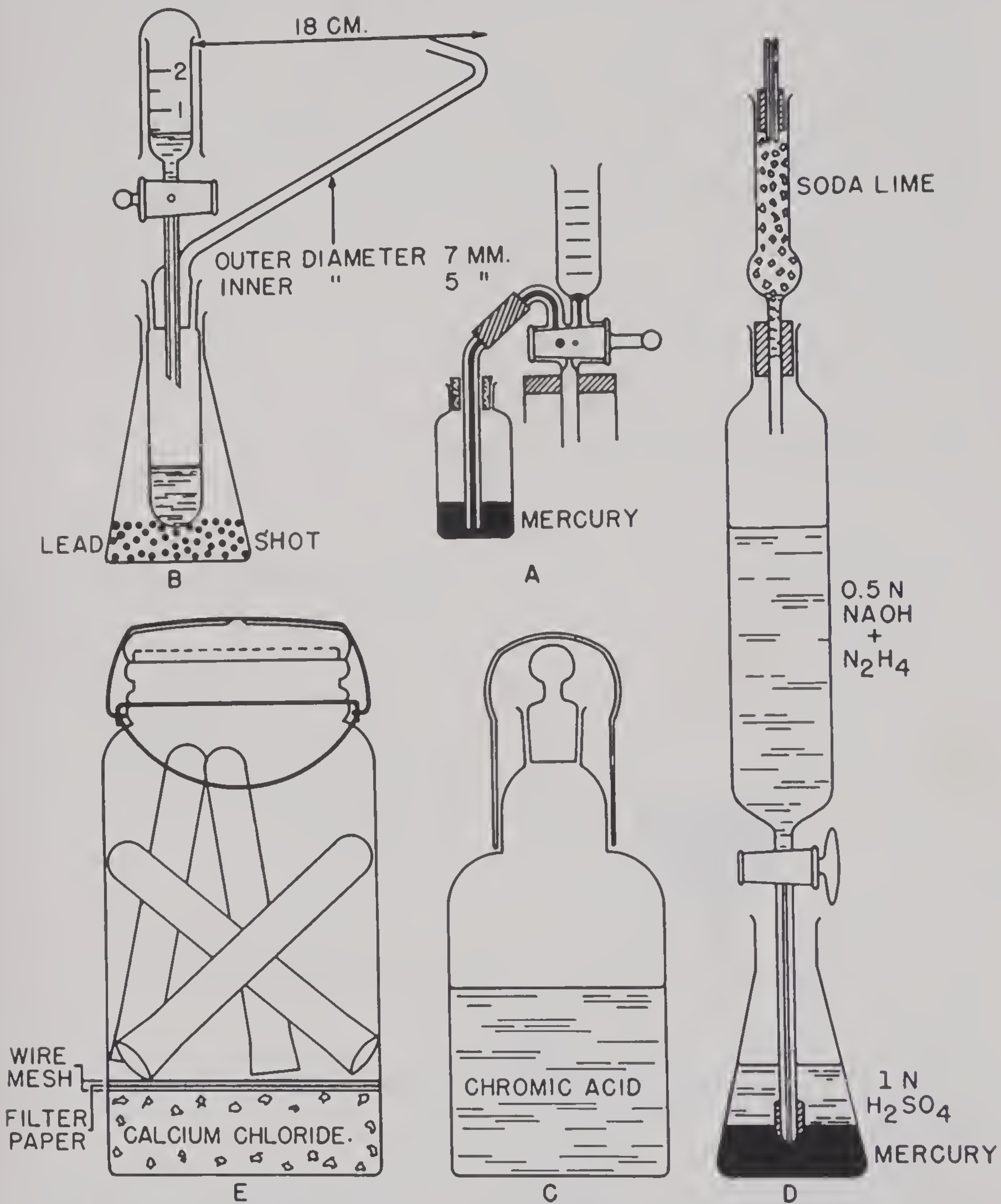

FIGURE 3. Accessory apparatus. *A*, small bottle of mercury arranged for sealing capillary of cock *b* (Fig. 1); *B*, Pyrex 100 cc. flask weighted with small lead shot serving as a stand for combustion tube after analysis; *C*, 100 ml. bottle provided with cover and ground joint to prevent access of moisture to combustion fluid; *D*, cylinder for holding alkaline hydrazine solution, hung with tip protected from atmospheric CO_2; *E*, fruit jar arranged for drying and storing washed combustion tubes. One quart jars serve for the 15 ml. tubes used for microcombustions, and two quart jars for the 25 ml. tubes used in macrocombustions.—After Van Slyke and Folch.[356]

Erlenmeyer flask, provided with a ground glass stopper, place 25 gm. of CrO_3, and 5 gm. KIO_3, followed by 167 ml. of syrupy phosphoric acid (sp. gr. 1.7) and 333 ml. of *fuming* sulfuric acid (20 per cent free SO_3). Leaving the stopper off, heat the mixture on a wire gauze until the temperature reaches 140–150°. Rotate the flask gently at times to assist solution of the chromic anhydride and escape of CO_2 formed by oxidation of any traces of organic matter that may be present. When 150° has been reached, remove the flame, cover the open flask with an inverted, lipless, 150 ml. beaker, and let the mixture cool to room temperature. When cool, insert the glass stopper, but also keep the inverted beaker permanently over the stopper to prevent dust from settling on the rim of the flask and contaminating the fluid as the latter is poured out. For use in analyses a portion of the fluid is poured into a protected bottle, of 100 ml. capacity, as shown in Fig. 3, *C*.

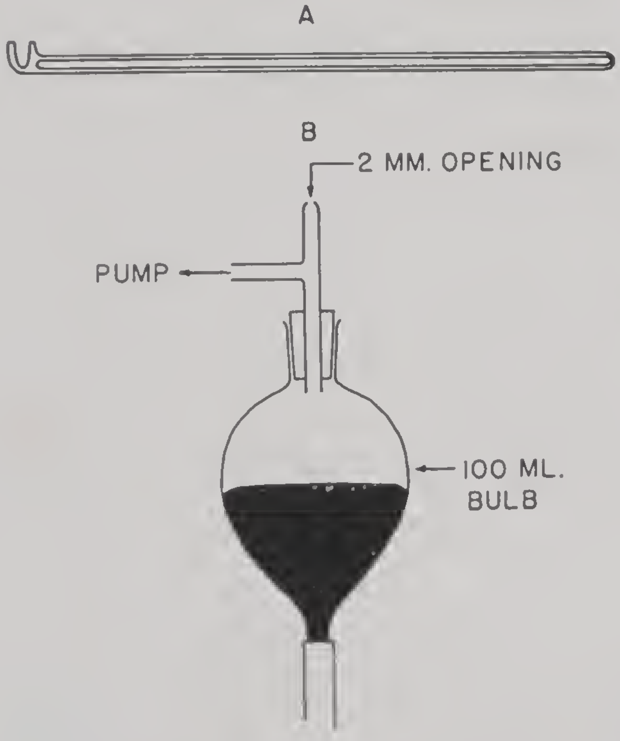

FIGURE 4. *A*, glass tubes for measuring 100 and 200 mg. of pulverized KIO_3. The inside diameter is about 4 mm. The measuring receptacle can be made somewhat oversize, and ground down to exact measure on an emery wheel. *B*, attachment of suction pump to leveling bulb to raise and lower the mercury in chamber *C* (Fig. 1) without moving the leveling bulb.—After Van Slyke and Folch.[356]

The fluid must be protected from absorption of water from the atmosphere, as water diminishes the efficiency for combustion. Besides its oxidizing efficiency, the anhydrous acid mixture has the advantage of a high solvent power for chromic acid: it dissolves over 25 gm. of CrO_3 per 100 ml. at room temperature.

Since the CrO_3 is hygroscopic and may vary in its moisture content, it is desirable to check the chromic acid concentration of the solution by titration. The chromic acid solution as made up, 5 gm. per 100 ml., would be nearly 0.5 *M* if the CrO_3 were anhydrous. The concentration shown by titration of our reagents has consistently been about 0.475 *M*.

If a solution as prepared is found to be weaker than 0.475 M, enough more CrO_3 should be added to bring it up to this concentration. The titration is carried out as follows:

Dilute a portion of the chromic acid solution to 10 volumes with water. Of the diluted solution measure 3 ml. into a 50 ml. Erlenmeyer flask, and add 5 ml. of water and 10 ml. of 10 per cent KI solution. After the flask has stood quietly for five minutes to complete the reaction between chromic acid and HI, the iodine produced is titrated with 0.1 N thiosulfate. (Ml. of 0.1 N thiosulfate used $\times 0.1$) = (molar concentration of chromic acid in the combustion fluid). This standardization should be repeated once a month, as the reagent loses strength on standing.

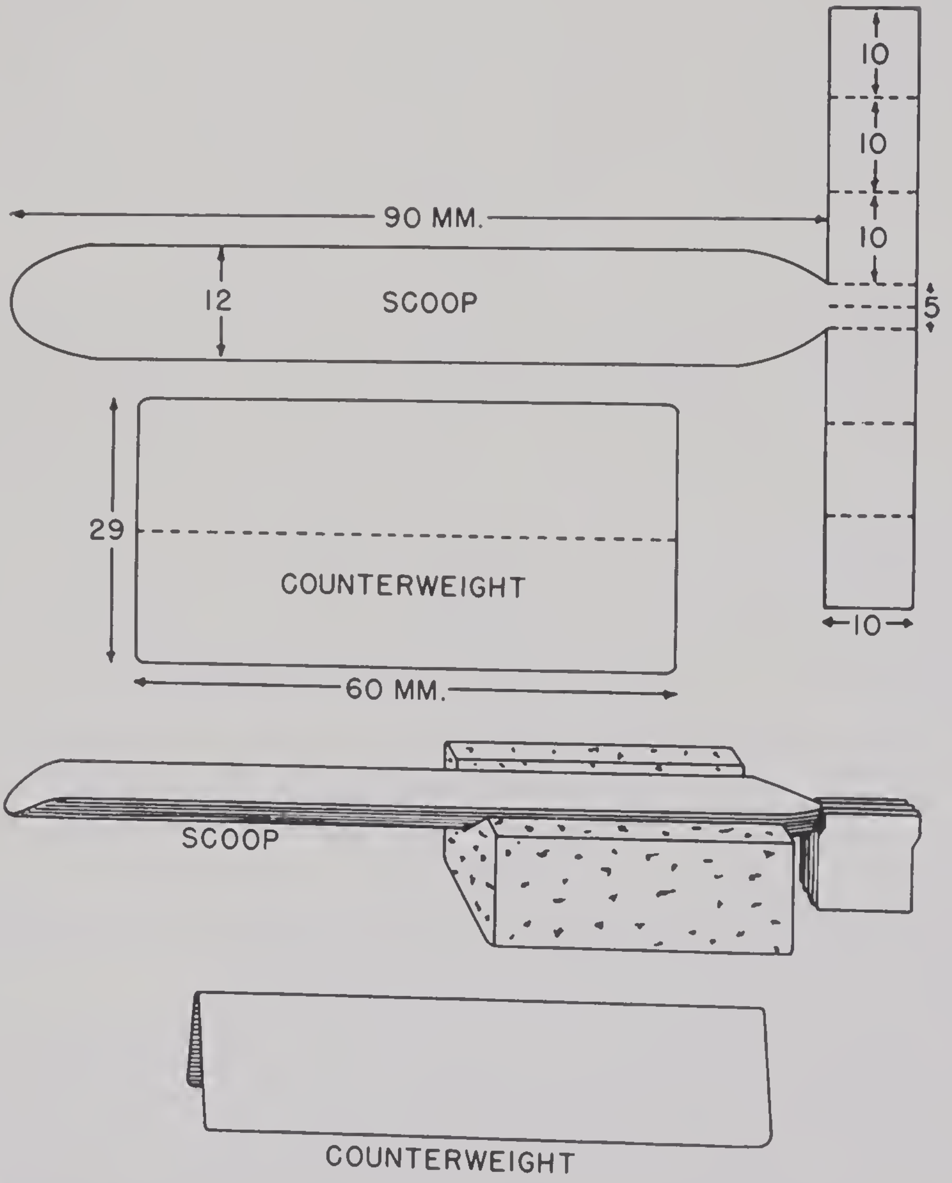

FIGURE 5. Sheet aluminum scoop and counterweight used in weighing samples for microcombustions. The aluminum used is of 0.25 to 0.30 mm. thickness. The upper drawing indicates the pattern in which the sheet metal is cut, with the dimensions in mm., and the lines of sharp bends shown by broken lines. The scoop is shaped in a half cylinder by bending it about a rod of 7 mm. diameter. The back portion, with the folded metal, serves as a handle, and as a weight centered below the scoop to insure the latter against overturning on the balance pan.—After Van Slyke and Folch.[356]

Potassium iodate, reagent grade, pulverized.

Approximately CO_2-free 0.5 *N* NaOH plus 0.3 *M* hydrazine. A solution of approximately CO_2-free NaOH of 0.815±0.005 *N* concentration is first prepared sufficient to last for a season's combustions. A liter or two of distilled water is acidified with a few drops of dilute acid and is boiled to expel CO_2. The water is cooled in a flask protected from atmospheric CO_2 by a soda lime tube, and enough concentrated (18 to 20 *N*) NaOH solution, from which carbonate has been allowed to settle, is added to bring the solution to somewhat over 0.815 *N* concentration. A portion is titrated, and the remainder is diluted with a volume of water calculated to bring the concentration down to 0.815 *N*. The solution is stored in a paraffin-lined aspirator bottle, of which the stopper is provided with a soda lime tube, while the outlet connects with a capillary glass tube of 2 mm. bore and 12 or 15 cm. length; the rubber tube connecting the capillary tube to the bottle's outlet is of walls about 3 mm. thick.

From this solution the alkaline hydrazine solution is made in portions sufficient only to last for about a month, as the hydrazine slowly decomposes. Into a volumetric flask one weighs 2 gm. of hydrazine sulfate for each 100 ml. capacity of the flask. The capillary outlet of the 0.815 *N* NaOH bottle is washed free of any adhering carbonate, and a little of the alkali solution is wasted, to wash out any carbonate that may have formed in the outlet. The outlet capillary is then inserted nearly to the bottom of the flask with the hydrazine sulfate, and alkali is run in until the flask is about two-thirds filled. Without withdrawing the delivery tube, the flask is whirled a few times to dissolve the hydrazine. Alkali solution is then added until the flask is filled to the mark. The flask is stoppered and the solution mixed. The solution is then at once drawn by suction into the cylinder shown in Fig. 3, *D*. The last fifth of the alkaline hydrazine solution is left in the volumetric flask, as this portion is more likely to be contaminated with atmospheric CO_2.

When not being used for delivery of alkali, the cylinder with the alkali-hydrazine solution is kept with its tip protected from CO_2 as shown in Fig. 3, *D*.

The hydrazine in the alkaline solution loses about half its strength on standing for a month under ordinary laboratory conditions. It is adequate until the loss exceeds this amount. When alkali-hydrazine solution is made up, it should be dated, and at the end of a month any that is left is discarded.

The hydrazine content can be determined gasometrically in a few minutes as follows: The alkaline hydrazine solution is diluted five-fold. 1 ml. of the dilute solution is measured with a stop-cock pipette into the chamber of the gas apparatus, and is followed by 1 ml. of saturated KIO_3 solution. The mercury in the chamber is lowered to the 50 ml. mark, and the chamber is shaken for two minutes to extract the N_2 formed by the reaction, $2NaIO_3+3N_2H_4=3N_2+2NaI+6H_2O$. The p_1 reading is taken with the gas at 2 ml. volume. A blank analysis is made with water in place of the diluted hydrazine solution, and the manometer reading is taken as p_0. The pressure P_{N_2} of N_2 from hydrazine is calculated as $P_{N_2}=p_1-p_0$. To calculate the molar concentration of hydrazine in the alkali reagent solution one multiplies P_{N_2} by five times the factor for a 1 ml. sample in Table 30 (fifth column) of Peters and Van Slyke,* or in Table III (seventh column) of Van Slyke and Neill.† (The fact that the S value stated in the tables is 3.5 ml., while it is 2 ml. in the hydrazine analyses, is without significant effect, because of the slight solubility of N_2.) The alkaline hydrazine solution as made up is 0.154 M in N_2H_4 concentration, and gives on analysis as described above a P_{N_2} of 280 mm. at 20°.

It is desirable to make the concentrations of NaOH and hydrazine sulfate exact, as they affect the solubility of CO_2 in the acidified reagent mixture from which the CO_2 is extracted at the end of the combustion, and the solubility affects the calculation factors.

2 *N* lactic acid. C.P. concentrated lactic acid (sp. gr. 1.20) is diluted with water to five volumes, and the concentration is checked by titration, with phenolphthalein as indicator.

Approximately 5 *N* sodium hydroxide. This is conveniently kept in a cylinder of the type shown in Fig. 3, *D*, but with no rubber ring about the delivery tip. The number of drops is determined in which 0.5 ml. is delivered.

Procedure

Placing sample in combustion tube: (a) *Samples weighed on microbalance:* It is desirable to deposit the weighed sample in the bottom of the combustion tube, *T* (Fig. 1), without scattering any of the material on the upper walls where it might escape complete combustion. The aluminum scoop shown in Fig. 5 has been found convenient both for weighing and for depositing the sample in the combustion tube. Scoop and counterpoise are balanced within 0.1 mg., the scoop being kept slightly the heavier. The scoop and its counterpoise are kept in the balance case between weighings. They are then ready at temperature equilibrium with the balance, and the sample can be added and weighed without delay.

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* Peters, John P., and Donald D. Van Slyke, Quantitative Clinical Chemistry, Vol. II, Methods, Williams and Wilkins Co., Baltimore, 1932.

† Van Slyke, Donald D., and J. M. Neill, *J. Biol. Chem.*, 61: 523, 1924.

The counterpoise is placed on the right-hand pan of the balance, and a sample of dried substance sufficient to contain preferably between 2.5 and 3.5 mg. of carbon is placed in the scoop, while the latter, in the balance case, rests on its cork support as shown in Fig. 5. The scoop is lifted onto the left balance pan by means of the pincers with jaws lined with chamois skin. The scoop and sample are weighed at once. If the material is slightly hygroscopic, one can repeat the weighings at intervals of one minute for three minutes and obtain the correct weight by extrapolating back to zero time. Material too hygroscopic to be treated in this manner must be weighed in one of the customary closed tubes used in microanalyses of such material, but we have rarely found them necessary.

To transfer the sample to the combustion tube the latter is taken from its jar (Fig. 3, *E*) and is held horizontally with a test tube holder while, with the chamois-protected pincers, the scoop with sample is inserted into the tube. Tube and scoop are then tilted to a vertical position, depositing the sample in the bottom of the tube. If portions of the sample adhere to the scoop, the latter is struck sideways gently against the tube to dislodge the material. If a slight amount remains adherent, it does not matter, as the weighing is by difference. If during these operations the combustion tube were held in the hand instead of a test tube holder, the warmth of the hand would penetrate into the tube and affect the scoop so that 3 minutes instead of 1 minute would be needed to regain temperature equilibrium after return of the scoop to the balance pan.

The emptied scoop is returned to the balance pan, and the balance is closed and let stand one minute for the scoop to regain temperature equilibrium. The empty scoop can be weighed at any time after this. We prefer, however, to make the second weighing within at most 10 minutes, in order to forestall the possibility of changes in the zero point of the balance. If the second weighing is made within 10 minutes, and the temperature of the balance room is reasonably constant, we have found it as a rule unnecessary to recheck the zero point of the balance.

Because of its freedom from static electricity, its light weight, and the quickness with which it reaches temperature equilibrium, the light aluminum foil has proved to be an ideal container for microweighings. We have on occasions finished a complete combustion, weighings included, within 20 minutes.

An alternative weighing procedure is to weigh the sample in a micro porcelain boat 15 to 17 mm. long, such as is used for microcombustions in furnaces. The boat with the sample is slid gently into the slightly inclined combustion tube, which is not turned vertically until the boat reaches the bottom. A sticky substance or non-volatile liquid can be handled in this manner.

The combustion tube with the sample is at once placed in a covered beaker, so that no opportunity is given for laboratory dust to fall into the tube.

(*b*) *Samples from evaporated solutions:* When submicrocombustions are carried out, with 0.3 to 0.7 mg. of carbon, the samples are usually too small to be weighed with 1/1000 accuracy on available microbalances, and must be measured into the combustion tubes as aliquots of solution, from which the solvent is then removed by evaporation. The concentration is so arranged that a sample of proper size will be contained in 1 or 2 ml. of solution. A 1 or 2 ml. pipette can be made to deliver an aqueous or alcoholic solution within 1 part per 1000. A weight burette, however, with a stem long enough to reach to the bottom of the combustion tube offers the most exact means of measuring the solution. It is desirable to use a volume of not more than 2 ml. in order not to have the solution run up the sides of the tube further than the combustion fluid to be added later.

To evaporate aqueous solutions in the combustion tubes the simplest and safest procedure is to leave the tubes overnight, somewhat inclined, in a large evacuated dessicator. The evaporation proceeds slowly, without danger of spattering, or of decomposition of heat-sensitive substances. Aqueous solutions of material which is not sensitive to heat can be evaporated by immersing the lower ends of the tubes in sulfuric acid in a Pyrex beaker which is heated on a steam bath, the top of the beaker being covered with a folded filter paper to keep out dust.

To remove volatile organic solvents, such as alcohol, ether, or acetone, the alundum pieces to prevent bumping are added (see *Apparatus*), and the tubes are placed in a beaker containing a 2 cm. layer of sulfuric acid. The top of the beaker is covered with a filter paper and the beaker is heated on a steam bath until all visible solvent is evaporated. The warm beaker is removed from the bath and placed in a desiccator, which is evacuated three times, air filtered free from dust being admitted after each evacuation. When alcohol is the solvent, add two or three drops of water to the residue before the beaker is placed in the desiccator.

It is essential that the tubes be not left on the steam bath longer than is necessary to drive off the visible organic solvent. The dry residue is left in such a thin film that it is pecularly accessible to oxidation by the air. Letting the tube heat for an hour after the film has dried may cause loss of one or two per cent of the carbon from a substance like cholesterol, which is ordinarily stable in air.

It is good practice to carry out the combustion within a few hours after the solvent has been evaporated. Even at room temperature some materials, when deposited in a thin film, appear to oxidize on standing, and yield low results when subsequently burned.

Connecting combustion tube with manometric chamber: Into the tube are dropped several pieces of the cleaned alundum or sand to insure smooth boiling, then 200 mg. of KIO_3 for a micro-, or 100 mg. for a submicroanalysis.[*] The KIO_3 may be measured by the tube in Fig. 4, *A*. With a medicine dropper a thick ring of syrupy phosphoric acid is then drawn around the upper part of the ground glass joint of *T* (Fig. 1), while *T* is in a horizontal position. Cup *F* of connecting tube *Q*, which has been resting as shown in Fig. 3, *B* in the combustion tube with which it was last used, is filled to the 2 ml. mark with fresh combustion fluid and the stopper is fitted to the combustion tube containing the sample to be burned. The combustion tube is now connected with chamber *C*.[†] as shown in Figs. 1 and 2, chamber *C* being completely filled with mercury.

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[*] If a series of submicrocombustions is to be carried out, it may be convenient to prepare enough chromic acid solution with predissolved KIO_3 for the day's analyses. KIO_3 in the proportion of 50 mg. per ml. of the chromic acid solution is added to the latter, and is dissolved by heating not higher than 160°. The solution after cooling is transferred to the bottle in Fig. 3, *C*. At room temperature the solution is several times supersaturated with iodic acid, but the latter will remain dissolved for a day before significant amounts crystallize out.

[†] For connecting tube *Q* to the capillary of *C* we use a thick tube of soft, elastic rubber; to fit over 7 mm. glass tubes the bore is 5 to 5.5 mm. and the walls are 5 to 6 mm. thick. With this the continual connecting and disconnecting are easier than with thin rubber, and the connection is more secure.

Preliminary ejection of air: Cock *b* (Fig. 1) is turned to connect *C* and *T*, and the mercury in *C* is lowered to the 50 ml. mark. This procedure draws about two-thirds of the air from *Q* and *T* over into *C*. Cock *b* is then closed, mercury is readmitted into *C*, the air trapped over the mercury in *C* is ejected through cup *E*, and cock *b* is closed again.

The ejection of the air from the system has two advantages. One is that removal of the air diminishes to insignificance the part of the blank that is due to atmospheric CO_2 present in the apparatus when the combusion is begun.

A second advantage is that when the CO_2 formed by combustion is transferred from the combustion tube to the alkali solution in *C* (Fig. 1), by running the gases back and forth between *C* and *T* as described later, the absence of the inert gases of the ejected air halves the number of excursions required for the transfer. The fewer the interfering molecules of inert gas the quicker the path of the CO_2 molecules to the absorbing solution; hence speed of CO_2 absorption by the alkali solution varies inversely as the proportion of inert gases mixed with the CO_2.

Measurement of alkaline hydrazine solution into manometric chamber: Two ml. of the alkali-hydrazine solution are measured from *D* and *C* through a mercury seal, as shown in Fig. 1. (The solution should be at the same room temperature as the water in the jacket of *C*.) The admission of the alkali is best regulated by opening cock *b* wide and controlling the flow of mercury through cock *a*. The admission is stopped when the mercury in *C* falls to a level about 1 mm. above the 2 ml. mark, as shown in Fig. 1. Then *D* is withdrawn and mercury from *E* is let into the chamber to fill the capillaries of cock *b*. The displacement of the slight volume of solution from the capillary of *b* into the chamber brings the volume of solution in *C* exactly to the 2 ml. mark. Cup *E* is rinsed with acidified water; this cup is never permitted to stand with its walls wet with alkali.

Combustion: *L* is lowered, and mercury is drawn out of *C* and the manometer until the mercury in the manometer is about level with the 2 ml. mark on *C*. Cock *a* is then closed, and cock *b* is turned to connect chamber *C* with the combustion tube. The leveling bulb is then placed, as shown in Figs. 1 and 2, at such a height that the mercury surface in it is about level with the 50 ml. mark on *C* and remains at this level until the combustion is finished. A measured volume of combustion fluid is now run from *F* into *T*; for microcombustions, with 2 to 3.5 mg. of carbon, 2 ml. of the fluid are used, while for the submicroanalyses with 0.3 to 0.7 mg. of carbon, only 1.5 ml. are taken. The micro flame is now brought under the combustion tube, and the arrangement is as shown in Fig. 2.

Fine bubbles of CO_2 gas begin to rise in the combustion fluid as soon as the latter is warmed. A minute or two is taken to warm the fluid to boiling. Preferably the CO_2 evolution should not be so rapid that it makes at any time a foam collar more than 2 cm. high on the fluid; although even if it should fill the greater part of the tube the analysis is not necessarily lost. After the initial CO_2 evolution the fluid is heated rapidly to boiling.

As CO_2 and O_2 are evolved, the mercury falls in *C* (Fig. 1) and rises in the manometer. Cock *a* is slightly opened every few seconds at this stage to admit mercury from *L* into *C* and keep the gas space in *C* at about 1 ml. Within about a minute from the beginning of heating enough gas has been evolved to press the mercury in the manometer up to its top, and to permit complete opening of cock *a* without causing backflow of alkali solution from *C* to *T*. Cock *a* is now left fully open during the rest of the combustion, and the boiling proceeds quietly at about 150 mm. less than atmospheric pressure. Vigorous boiling at about 600 mm. pressure is continued, with foam filling one-third to one-half of the tube, for 1.5 minutes to complete the combustion. Of the substances analyzed we have not found any which required more than one minute's vigorous boiling for complete combustion. Even the fatty acids, usually among the more resistant substances in combustion, were burned completely in one minute, after the pressure had reached 600 mm. With many substances combustion is complete as soon as this point is reached.

Making the boiling vigorous is emphasized because it has been found essential with palmitic and stearic acids. With most substances gentle boiling suffices. The higher fatty acids, however, as the combustion fluid is warmed, melt and form a film on the surface. It is perhaps because of this behavior that vigorous boiling is essential with them. Gentle boiling (with a foam collar under 5 mm. high) may yield results 0.5 to 1 per cent too low with palmitic and stearic acids.

It is unnecessary and undesirable to continue the boiling longer than 1.5 minutes. By the end of this time the chromic acid has evolved practically all of its labile oxygen, and the iodic acid begins to decompose and give off iodine and oxygen. If heating were continued until all the iodic acid had been decomposed, the volume of additional O_2 evolved would retard appreciably the absorption of CO_2 next described, so that 25 instead of 20 excursions of the mercury in *C* (Fig. 1) might be required for complete absorption. If the period of boiling at 600 mm. pressure is kept under two minutes, so little iodic acid is decomposed that the iodine vapors are barely visible in the combustion tube.

In case any particles of the sample have been dropped on the upper parts of the combustion tube, or in case the foaming during the initial CO_2 evolution has been so rapid that the foam reached above the half-way mark of the tube, it is a desirable precaution, after boiling at 600 mm. has been established, to shake the combustion tube so that the oxidizing liquid will reach any unburned material in the upper part of the tube. Or the flame may be increased for a few seconds so that the boiling fluid reaches the upper part of the tube.

Absorption of CO_2 by alkali in gas chamber: After the combustion is completed, the flame is left under the tube exactly as during the combustion, while the mercury in *C* (Fig. 1) is lowered and raised 20 times to cause complete transfer of CO_2 to the alkali solution in *C*. The 20 excursions should take about three minutes. At each lowering of the leveling bulb the mercury in *C* is dropped to about the 50 ml. mark, and the fluid in *T* boils vigorously. The tube should then fill with foam. At each raising the bulb is lifted till the gas space in *C* is compressed to about 5 ml. After 5, 10, 15, and 20 excursions of the mercury in this manner, the respective percentages of the CO_2 transferred to the alkali solution in *C* have been found to be 91, 98.3, 99.7, and 100 respectively.

The volume of O_2 evolved from the reagents can be roughly estimated as follows: After the CO_2 has been absorbed, but while the combustion tube is still connected and the flame is still under the combustion tube, one sets the mercury meniscus in the chamber at the 50 ml. mark, closes cock *a* (Fig. 1) for a moment, and notes the height of the mercury in the manometer. If it does not lie more than 350 mm. above the mercury surface in *C*, the amount of O_2 present is not enough to prevent complete absorption of the CO_2 by 20 excursions of the mercury (see the next paragraph). If by unduly long boiling of the reagents, or leakage of air into the apparatus, the non-CO_2 gases exert a pressure greater than 350 mm., it is well to use a few more excursions to make certain of 100 per cent absorption.

When many combustions are carried out, it will be found convenient to avoid the labor of the many raisings of the mercury bulb by alternately applying and releasing suction at the top of the bulb, without moving the bulb from its usual level shown in Fig. 2. A good water aspirator will raise and lower the mercury at about the same rate as lifting the bulb by hand. A simple device for releasing the suction is shown in Fig. 4, *B*. With the pump connected directly, without an intermediate safety flask, suction is obtained by merely closing the open tube by a flap of chamois skin and is released by removing the closure. Or a three-way cock may be substituted; this is desirable if the suction tube is connected to an evacuated reservoir, in which it is preferable not to release the vacuum when the latter is released in the leveling bulb. When the suction method is used, it is desirable to have a mercury bulb of not over 100 ml. capacity, and to have sufficient mercury in the system so that the bulb will be nearly filled when all the mercury is withdrawn from *C* (Fig. 1). If there is a large gas space in *L*, it is more difficult to control the excursions of the mercury in *C*.

After absorption is completed, the flame is removed from the combusion tube, cock *b* (Fig. 1) is closed, and tube *Q* is disconnected from the chamber. The hot combustion tube is taken with tongs or a strong metal test-tube holder, and stood in a flask, as shown in Fig. 3, *B*.

Ejection of unabsorbed gases: Before O_2 and N_2 are ejected, the curved inlet capillary above cock *b* is filled with mercury drawn in from a small bottle as shown in Fig. 3, *A*. About as much mercury is left in this capillary above the cock as is shown in Fig. 1.

Then, with cock *b* closed, the gases in *C* are put under positive pressure by raising leveling bulb *L* a little above cock *b*. With the bulb at this level, cock *a* is closed and *b* opened to connect chamber *C* with cup *E* above it. Mercury is then admitted from *L* into chamber *C* until the rising alkali solution, driving the gases out through *E*, just reaches the bottom of cock *b*. In succession one then closes cocks *a* and *b*. The leveling bulb is lowered to the position shown in Figs. 1 and 2, and a little mercury is admitted from cup *E* into the chamber to seal the connecting capillary. A small bubble of air, trapped in the capillary, is thus readmitted into *C*, but it has no influence on the CO_2 determination, since it is CO_2-free.

Extraction of CO_2 and reading of p_1: Exactly 1 ml. of 2 *N* lactic acid is measured into chamber *C* from an accurate stop-cock pipette provided with a rubber-ringed tip. The admission is made through a mercury seal in the same manner shown in Fig. 1 for admission of alkali. Mercury to fill the connecting capillary between *E* and *C* is admitted after the acid.

The mercury in the chamber is then lowered to the 50 ml. mark, cock *a* leading to the leveling bulb is closed, and the chamber is shaken 20 or 30 seconds, extracting most of the CO_2 from the solution. The CO_2 that has entered the gas phase increases the pressure there enough to force the mercury down below the 50 ml. mark. To correct this displacement, enough mercury is admitted from the leveling bulb to bring the top of the mercury meniscus in the chamber exactly to the 50 ml. mark. The chamber is then shaken for 1.5 minutes to complete extraction of CO_2 from the solution.

Mercury is now admitted from the leveling bulb until the volume of the gas phase in the chamber is reduced, to 10 ml. for the microcombustions with 2 to 3.5 mg. of carbon, or to 2 ml. for the submicrocombustions with a fifth as much carbon. . . . The chief precautions are to complete the admission of the mercury within a space of 30 or 40 seconds, and to avoid setting the mercury in the chamber and manometer to oscillating by jerky opening or closing of cock *a*. If these precautions are followed, the reabsorption of CO_2 that occurs during the admission of the mercury is so constant (estimated as 0.7 per cent when the gas is compressed to 10 ml., 1.6 when it is compressed to 2 ml.) that variations in reabsorption affect results by not more than one part per 300 when the gas is brought to 2 ml., or one part in 1000 when it is brought to 10 ml. As the meniscus of the solution in the chamber approaches the 2 or 10 ml. mark it is desirable to watch it with a hand lens, in order to stop it exactly on the line.

The reading, p_1, is then taken on the manometer. If it is desired to check this reading, the mercury in the chamber is lowered again to the 50 ml. mark, shaken for a minute, and the gas is returned to 2 or 10 ml. volume for repetition of the p_1 reading. With practice it will be found that the duplicate readings check so closely that the repetition is unnecessary.

Reabsorption of CO_2 and reading of p_2: After the p_1 reading has been taken, the cock leading to the leveling bulb is opened, while the bulb is left at the level shown in Figs. 1 and 2, so that the gas in the chamber is under slight negative pressure. Into cup *E* one measures 0.5 ml. of the 5 *N* sodium hydroxide solution; the measurement is most conveniently made by counting the drops. The alkali is then admitted into the chamber, with care not to admit any air into either the chamber or the capillary above cock *b*. In the submicrocombustions, with less than 2 ml. of CO_2 present, absorption of all the CO_2 occurs about as rapidly as the alkali can be run in. When the larger amounts of CO_2 measured at 10 ml. volume are present, however, it takes a little longer to complete the absorption, and one admits the alkali over a period of a half minute or more. When all the alkali except enough to fill the capillary below the cup is in the chamber, one pours 2 or 3 ml. of acidified water into the cup, followed by about 0.5 ml. of mercury. The mercury is then run into the chamber, dislodging any of the alkali solution which may have stuck in the part of the chamber under cock *b*.

Sometimes the somewhat viscous 5 *N* alkali solution flows in a solid column into the 4 mm. tube at the top of the chamber, instead of streaming down the walls and absorbing the CO_2. In this case one follows the alkali with about 1 ml. of mercury, which is admitted in several fine jets. These dislodge the alkali and cause quick absorption of the CO_2.

To mix the solutions in the chamber, and to insure absorption of the last traces of CO_2, one now lowers and raises the mercury in the chamber three times, each lowering bringing the surface of the solution in the chamber, not to its bottom, but only to a point a little below the 10 ml. mark, and each raising bringing the pressure up to about atmospheric.

The solution meniscus in the chamber is then brought to a point a little below the 2 or 10 ml. mark at which p_1 was read, and is let stand there for 1 minute while the solution drains down from the walls above the mark. During this minute one can read the temperature in the water jacket of the chamber and look up the corresponding carbon factor. Then the meniscus is raised exactly to the 2 or 10 ml. mark and reading p_2 is made on the manometer. The pressure, P_{CO_2}, of CO_2 from the combustion is calculated as $P_{CO_2} = p_1 - p_2 - c$, where c is the value of $p_1 - p_2$ obtained in a blank analysis.

Washing chamber after analysis: To clean the chamber for the next analysis the used alkaline lactate solution is ejected and the chamber is washed once with dilute acid and once with water. A rapid and convenient technique is the following. The mercury leveling bulb is lowered about 80 cm. below chamber *C* (Fig. 1) so that the mercury will all drain out of the chamber. While it is draining, a few drops of the 2 N lactic acid are placed in the cup at the top of the chamber, together with enough water to fill the cup. The acidified water (but no air) is now let into the evacuated chamber. The leveling bulb is then raised and the solution expelled from the chamber. The washing is then repeated in the same manner, except that only distilled water is used. After the water is ejected, the mercury is lowered once more to the bottom of the chamber, and is allowed to rise rather slowly, so that the film of water adherent on the sides of the chamber is detached and floats up on the mercury. The drop of water thus collected, together with about 1 ml. of mercury, is run up into the cup, and the apparatus is ready for the next combustion.

Alternative rapid analysis without reabsorption of CO_2: This procedure is slightly less exact, but saves three or four minutes on each analysis, and serves well when series of analyses are required, as in routine blood fat determinations, in which an increase of a mm. in the error of P_{CO_2} is not important.

The analysis is carried out, as described above, until the p_1 reading has been taken, and this reading finishes the analysis. P_{CO_2} is calculated as $P_{CO_2} = p_1 - p_0$, where p_0 is the p_1 reading obtained in a blank analysis at the same temperature. If the blank analysis is performed within 3° of the same temperature as the unknown, the blank reading can be corrected to apply to the unknown by calculating p_0 as (p_1 of the blank) + (rise in p_{H_2O}), where the "rise in p_{H_2O}" is the increase in vapor tension of water caused by the temperature change from the temperature of the blank analysis to the temperature of the substance analysis; *e.g.*, if the blank is run at 20°, where the vapor tension of water is 17.4 mm., and the carbon analysis is run at 22°, where the vapor tension is 19.6 mm., the p_0 used is the p_1 of the blank plus 2.2 mm. If the temperatures were reversed, the blank being at 22° and the unknown at 20°, the p_0 used would be the p_1 of the blank minus 2.2 mm., the "rise in p_{H_2O}" being negative. For convenience in correcting p_0 values for temperature changes the vapor tensions of water are given in the last column of Table I.

Between analyses performed without reabsorption of CO_2 it is necessary to wash chamber *C* (Fig. 1) only once, with water, since the amount of CO_2 in the film of acid solution left after the reagents have been ejected from the chamber is slight.

TABLE I

FACTORS FOR CARBON CALCULATION

Temperature	Factors		Vapor tension of water
	Submicroanalysis $a = 2.00$ $S = 3.00$ $i = 1.016$	Microanalysis $a = 10.00$ $S = 3.00$ $i = 1.007$	
°*C.*			*mm.*
10	0.001474	0.007303	9.1
11	66	265	9.8
12	58	228	10.4
13	51	192	11.1
14	44	157	11.9
15	37	122	12.7
16	30	088	13.5
17	24	054	14.4
18	17	020	15.3
19	10	0.006987	16.3
20	03	954	17.4
21	0.001397	922	18.5
22	90	890	19.6
23	84	859	20.9
24	78	828	22.2
25	72	798	23.5
26	66	769	25.0
27	60	740	26.5
28	54	711	28.1
29	49	683	29.7
30	43	655	31.5
31	37	628	33.4
32	32	601	35.3
33	27	575	37.4
34	21	549	39.5
35	16	523	41.8

When determining total plasma lipids by combustion, following Van Slyke, Page, and Kirk [*J. Biol. Chem.*, 102: 635, 1933], one may multiply the carbon factors by 1.266 in order to obtain factors for calculation of mg. of total lipids directly from P_{CO_2}.

When cholesterol is determined by combustion of the digitonide, $C_{27}H_{46}O \cdot C_{55}H_{94}O_{28}$, one may multiply the factors of Table I by 0.3926, in order to obtain a table of factors for calculating mg. of cholesterol from P_{CO_2}. [Factors for macroanalysis have been omitted from the original table of Van Slyke and Folch.[356]]

Calculation of Results of Micro- and Submicroanalyses

The mg. of carbon in the sample are calculated by the factors of Table I, corrected if necessary by a factor obtained from control analyses of a standard pure substance, as described later.

$$\text{Mg. carbon} = P_{CO_2} \times \text{factor}$$

With any apparatus in which the 2 and 10 ml. marks are as accurate as in most chambers furnished by the better manufacturers, the factors in Table I can be used unchanged for many routine purposes. For combustions of assured accuracy, however, it is desirable to control all constants together by determining the *b* value and with it preparing one's own table of factors by correction of those in Table I. [For method of determining the "*b*," factor the reader is referred to the original method.]

CYTOMETRIC AND HEMATOCHEMICAL METHODS

THE methods presented on the following pages provide for: the quantitative estimation of hemoglobin and the formed elements of the blood (red and white blood cells, and platelets); measurement of the physical characteristics of the erythrocyte; and, the determination of mineral and lipid constituents of whole blood, plasma, serum, erythrocytes, leucocytes, platelets, and posthemolytic residue (stroma) of red blood cells. Since the primary objective of this laboratory has been to study growth changes, we have emphasized the blood procedures which offer the greatest possibilities of detecting progress in the maturation of normal children (See p. 27). The limitations of the metabolic balance technique have precluded the use of some procedures which would have been informative. All of the methods are subject to gross influences from minute defects in equipment and slight irregularities in procedure. Accurate sampling and analytical techniques, under well-controlled and standardized conditions, are essential to obtaining comparable hematological data.

CYTOMETRIC DETERMINATIONS[*]

Capillary Blood Sampling

Blood obtained from a finger-puncture is used for red blood cell counts and corpuscular measurements, total and differential white cell counts, and platelet counts. Wipe off the finger tip with ether (not alcohol) to make it hyperemic, and puncture the skin with a No. 11 Bard-Parker knife so that a free blood flow is established. Samples of blood should be obtained with pipettes certified by the United States Bureau of Standards.

Hemoglobin determination: Dilute whole blood sample 1:10 with 0.1 *N* HCl in a white cell pipette and allow to stand 30 minutes. Compare with standard scale of the Haden-Hausser hemoglobinometer according to directions which accompany instrument. Result is obtained in grams of hemoglobin per cubic millimeter of blood.

Blood cell counts: Draw fresh blood into a white cell pipette and dilute to 1:20 with 2.5 per cent acetic acid. Draw fresh blood into a red cell pipette and dilute to 1:200 with Hayem solution.[†] Shake the pipettes for 10 minutes in an angular-rotary shaker before charging the hemocytometer. Counts may be made with the Levy-Hausser, double chamber hemocytometer with improved Neubauer ruling. One chamber of the instrument is filled from the white cell pipette; the other from the red cell pipette.

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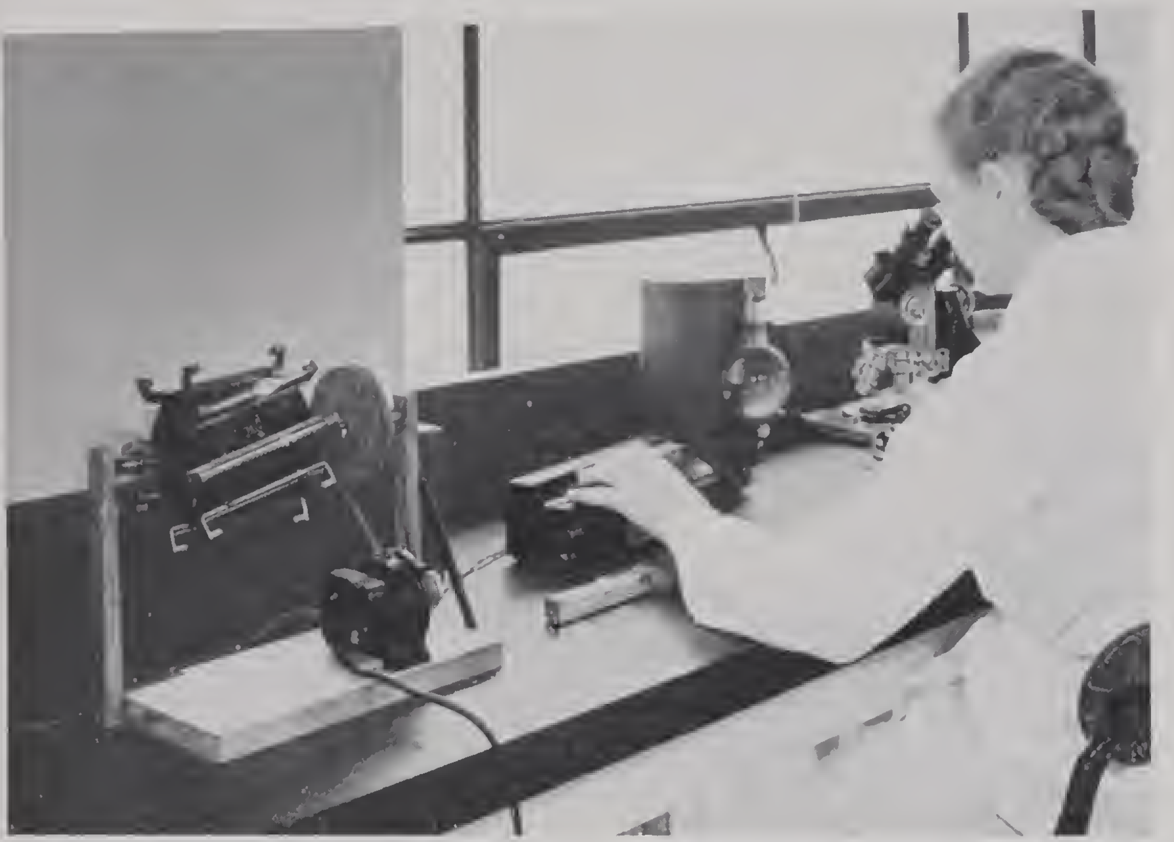
[*] Whenever venous blood samples were taken the cytometric determinations were made upon these samples, rather than upon capillary samples.

[†] Hayem solution: 5 gm. sodium chloride, 25 gm. of sodium sulfate ($Na_2SO_4 \cdot 10H_2O$) and 2.5 gm. mercuric chloride in water to make one liter of solution.



MECHANICAL ANGULAR-ROTARY SHAKER

—*Reproduced by courtesy of the* American Journal of Diseases of Children

The counts should be made according to published counting technique available with the instrument.*

Reticulocyte and platelet counts; differential white blood cell counts: Prepare two coverslips by dipping into a saturated alcoholic solution of brilliant cresyl blue and allow them to dry. At the same time prepare two coverslip smears with fresh blood from the finger-puncture. Prepare two other smears, using clean, dry coverslips. Set the four smears aside until dry. Apply Wright's stain† and leave on the smears for two minutes. Dilute with distilled water containing a drop of Sorenson's phosphate buffer mixture, *pH* 6.468,* for three minutes; wash off with distilled water and blot dry. Mount coverslips on microscope slides with Shillaber's non-drying immersion oil or cedar oil and observe under oil immersion lens.

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* *An improved technique for the counting of blood corpuscles, blood platelets, cells in cerebro-spinal fluid and pus cells in urine.* Technological Service Publ. DU 15. Arthur H. Thomas Co., Philadelphia, 1940.

† Wright's stain: Dissolve 1.5 gm. Wright's stain powder in 500 ml. of methyl alcohol. The solution must be filtered twice before using. Since different preparations of stain vary greatly in quality, the staining technique must be adapted to the properties of the staining solution used.

From the smear, prepared with brilliant cresyl blue, estimate the reticulocytes in terms of percentage of red cells and calculate the number of platelets upon the basis of the total red blood cell count. For the reticulocyte count, it is convenient to count 50 red cells in each of ten fields and the number of reticulocytes in the corresponding areas. At least 1000 red cells and the platelets in the same areas should be counted to calculate the total number of platelets by the formula:

$$\frac{\text{Red cells counted}}{\text{Total red cell count}} = \frac{\text{Number of platelets counted}}{\text{Total platelet count}}$$

With the smears prepared with clean coverslips make a differential count of 200 white blood cells and designate the polymorphonuclears according to Schilling's classification of myelocytes, juveniles, stab nuclears, and segment nuclears.

Mean diameter of erythrocyte:† Two margin-free smears (Schilling[290]) are necessary for determination of the mean diameter of the red blood cells. From the finger-puncture, pick up a fresh drop of blood with the edge of a coverslip. Touch the specimen edge of the coverslip to the face of a microscope slide, about one-half inch from one end, at an angle of 45°. Allow the blood to spread along the edge of the coverslip; then draw the coverslip toward the other end of the slide in a firm, even and rapid movement, so that the blood follows without crushing. A satisfactory preparation is made from a drop of blood which will make a smear one-third to one-half the length of the slide.

Select a "usable area" in the unstained blood film by microscopic examination, an area where the cell edges are touching each other without appreciable overlapping. Place the microslide on the table of the erythrocytometer, film side up, so that the "usable area" completely covers the aperture. An unstained blood film on a high-grade glass slide shows two distinct, sharply defined, spectral halos within the movable cylinder. The outer red bands are used for measurement. A black line should not be present between the two red halos, at the reading point, nor should there be any difference in the color intensity of the contiguous red bands. By lifting and depressing the inner sleeve, which is held at any desired level by the set screw, two well-defined, red halos are brought together without overlapping, and the measurement recorded from the position of the set screw on the scale. Four readings should be taken in different locations within the usable area of each smear. The total of the eight readings, in microns, divided by eight, represents the average diameter of a single erythrocyte.

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* Clark, W. Mansfield. The determination of hydrogen ions. The Williams and Wilkins Co., Baltimore, 1928. p. 210. (cf. Ref. 49)

† Sharp, E. A., and E. M. Schleicher. Bock's erythrocytometer and technic for its use. *J. Lab. Clin. Med.*, 21: 975, 1935. (cf. Ref. 304)

Venous Blood Sampling

Draw 50 ml. of blood from an arm vein into a 50 ml. syringe and eject immediately into glass receptacles prepared for the samples to be used in the various determinations. Serum, plasma or whole blood may be used in determinations of lipid and mineral content. If whole blood or plasma is to be used the entire sample may be heparinized in 15 ml. conical centrifuge tubes. After adding the blood to the tubes containing heparin the contents of the tubes should be stirred with an applicator until the heparin has been dissolved.

Preparation of serum: Serum is best prepared for mineral analyses by injecting the fresh venous blood beneath the surface of a layer of paraffin oil and centrifuging to separate serum from the clotted material.

Preparation of plasma: Plasma is prepared by centrifuging the heparinized whole blood at 2000 to 2500 R.P.M. for 45 minutes to one hour* and removing the plasma with a pipette without disturbing the cells in the bottom of the tube.

Preparation of white blood cells and platelets: After removing the plasma from the centrifuged, heparinized blood sample, the leukocyte layer containing almost all the platelets may be removed from the red cells with clean forceps and placed in a weighed watch glass. Adherent plasma and red cells should be removed with strips of clean filter paper before the watch glass containing the white cells and platelets is weighed. If determinations upon white blood cells alone are desired, the method given for preparing platelets for lipid analysis may be executed and the white cells then separated from the red cells.

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* Less than one per cent of the original number of platelets will remain in suspension in the plasma—the separation of platelets from the plasma must be complete or too high values for plasma lipid will result.

Preparation of red blood cells: After the plasma and the covering layer of white cells and platelets have been removed from the centrifuged, heparinized blood, a pipette, with the top sealed, may be passed through the packed erythrocytes and two-thirds to three-fourths of the red cells removed from the bottom of the tube.

Water content of cells: Samples of packed cells may be weighed on a microbalance in small, flat weighing bottles containing sand. The water lost is calculated after the material has dried for 48 hours at 85° C.

Specific gravity of blood cells: The specific gravity of cells may be calculated from the values for plasma and whole blood, as determined by weighing in pycnometer bottles on the microbalance. The *relative specific gravity* or density of the cells is calculated by the following formula:

$$\text{Sp.gr. of Cells} = \frac{100\ (\text{Sp.G. of whole blood}) - S\ (\text{Sp. gr. of plasma})}{C}$$

where C is the percentage of cells in the whole blood and S the percentage of plasma.

Preparation of Extracts for Lipid Analysis

Alcohol-ether extracts of plasma, serum, white blood cells and platelets, and red blood cells, may be prepared by the procedure given in the following paragraphs. After the lipids have been extracted by the alcohol-ether, the extracts may be made up to volume and then filtered; or, filtered into a volumetric flask, the precipitate washed twice with solvent and pressed to force out as much retained solvent as possible and the filtrate made up to volume. (Similar results are given by these alternative procedures. The second method of making to volume after filtration has certain theoretical advantages and a practical advantage in that Erlenmeyer flasks, in which the proteins are precipitated in the alcohol-ether, may be cleaned more readily than long narrow-necked volumetric flasks.)

When it is desirable to purify further the alcohol-ether extract of lipids, an aliquot (usually 50 to 100 ml.) of the alcohol-ether extract is evaporated to 1 ml. or less (to a gummy consistency) in a vacuum still in a stream of nitrogen below 50° C. The alcohol-ether residue is extracted with successive portions of boiling petroleum-ether, decanting the extracts through fat-free filter paper into a volumetric flask (50 to 100 ml.). After cooling, the petroleum-ether extract is made to volume.

Plasma or serum:* Pipette one volume of plasma or serum, slowly, into at least 20 volumes of alcohol-ether (3:1) in an Erlenmeyer or volumetric flask. Let stand one hour or over night. Extraction is complete without heating. (This is the method of extraction by cold dilution and is the simplest method yet devised of obtaining complete extraction of lipids from blood.)

Plasma extracts free of non-lipid extractives:† Add to one volume of plasma in a centrifuge tube, in succession, with stirring, 15 volumes of water, 1.25 volumes of colloidal iron solution (Merck's "Dialyzed Iron," containing five per cent Fe_2O_3) and 0.65 volume of a 1:1 aqueous solution of $MgSO_4 \cdot 4H_2O$. Centrifuge the precipitate for five minutes, then wash by centrifugation with 15 volumes of water plus 0.65 volume of the 1:1 $MgSO_4 \cdot 4H_2O$ solution. For routine analysis two washings suffice. Transfer the washed precipitate to a volumetric flask, marked to contain 10 times the volume of the plasma sample, using, successively, two volumes of absolute alcohol, one volume of alcohol, one volume of alcohol, two volumes of ether, and two volumes of ether, finally filling to the mark with ether. Filtration provides a clear solution containing all the plasma lipids without non-lipid extractives.

White blood cells and platelets:* Grind the weighed sample of white cells and platelets with cleaned sand and transfer from the watch glass with 1 to 2 ml. of water, into 25 volumes of alcohol-ether. Stir well to produce suspension in the extracting solution.

Red blood cells:* Shake one volume of accurately weighed or measured erythrocytes with an equal volume of distilled water in an Erlenmeyer or volumetric flask until hemolyzed. Add 25 volumes of alcohol-ether and shake the mixture vigorously for several minutes. With a clean stirring rod break any clumps which form on standing. The extract of the red blood cells must not be heated before filtering, since heat causes colored decomposition products to dissolve in the solvent and these contaminate the subsequent analyses and give false, high lipid values.

Platelets:‡ Draw a post-absorptive blood sample (usually 50 ml.) from an arm vein into a syringe containing 10 ml. of chilled anticoagulant solution. Deliver the blood into an ice-cold flask and adjust to twice the original volume by adding anticoagulant solution.* Centrifuge the diluted blood in chilled, 15 ml. conical tubes for 10 minutes at 800 R.P.M. Remove the diluted plasma containing some red and white cells, place in chilled tubes and centrifuge again at low speed for five minutes. By this time the platelet suspension can be decanted, leaving a small sediment of red and white cells. Repeat the centrifugation for five-minute periods, and decantation into clean, chilled tubes, 10 to 15 times, until no visible red cell sediment is thrown down. Separate the platelets from the suspension by high-speed centrifugation (2000 R.P.M.) for two hours. Wash the white sediment twice with chilled anticoagulant solution (diluted 1:2) by shaking and recentrifugation, and then dry *in vacuo* at 70° C.

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* Boyd, E. M. The oxidative micro-estimation of blood lipids. *Am. J. Clin. Path.*, 8: 77, 1938. (cf. Ref. 32)

† Folch, Jordi, and Donald D. Van Slyke. Preparation of blood lipid extracts free from non-lipid extractives. *Proc. Soc. Exper. Biol. Med.*, 41: 514, 1939. (cf. Ref. 102)

‡ Erickson, Betty Nims, Harold H. Williams, Ira Avrin, and Pearl Lee. The lipid distribution of human platelets in health and disease. *J. Clin. Inves.*, 18: 81, 1939. (cf. Ref. 83)

About 10 mg. of dried platelet material may be secured from each individual 50 ml. sample of blood. Approximately 50 mg. of dried platelets are required for each nitrogen and lipid distribution analysis. The lipids are extracted by suspending the platelet sample in 25 ml. of alcohol-ether solution over night, filtering the extract through fat-free filter paper (Whatman #43) into a 100 ml. volumetric flask, extracting the residue first with 30 ml. of ethyl alcohol (in a Soxhlet or similar type extractor) and then with 30 ml. of ethyl ether. The extracts are combined with the original extract in the volumetric flask and made up to volume. Nitrogen is determined on the dried, lipid-free, platelet material by the micro Kjeldahl method (p. 321).

Cell volume (Hematocrit): Fill two Wintrobe hematocrit tubes with heparinized whole blood and cover the tubes with a rubber cap. Centrifuge for 45 minutes at 2400 R.P.M. Read, on the graduated scale of the tubes, the total volume and the volume of red cells. Calculate the percentage volume of red cells. From the red cell count per 100 ml. blood (on the same sample) and the percentage volume of red cells, calculate the mean volume per cell.

$$\text{Mean cell volume of normal blood} = 87 \text{ cubic microns}$$

$$\text{Mean corpuscular volume} = \frac{\text{Vol packed red cells in milligrams per 1000 ml. blood}}{\text{Red cells in millions per cubic millimeter.}}$$

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* A sodium metaphosphate solution (2.0 per cent sodium metaphosphate, 1.0 per cent sodium chloride, and 0.2 per cent dextrose) proved the most satisfactory as a platelet preservative solution.

Volume thickness index of red blood cells: Haden* has published the three-dimensional chart of Van Boros by which the thickness of an erythrocyte can be determined directly from the diameter and corpuscular volume (Figure 1) but has designated

CHART FOR CALCULATING THE THICKNESS OF THE ERYTHROCYTE

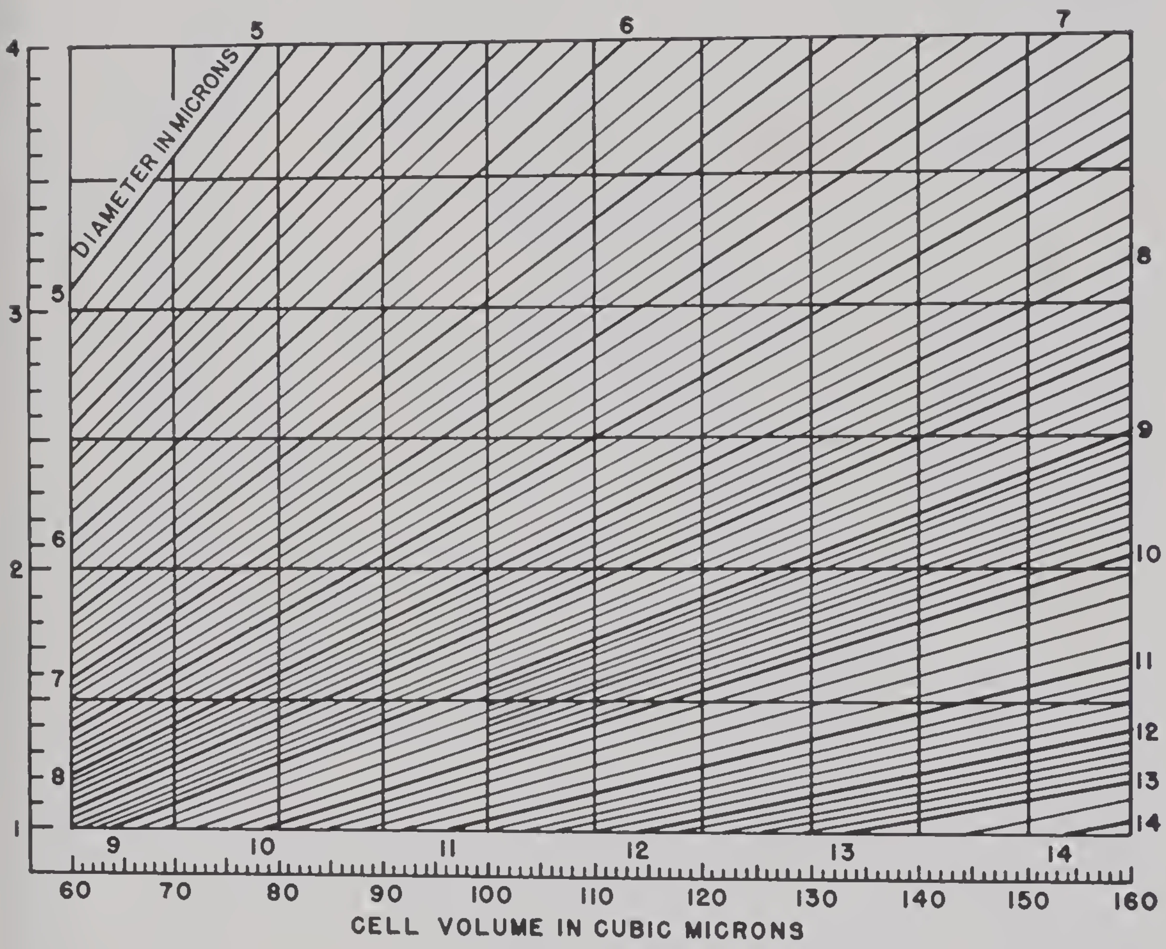

FIGURE 1. (After Haden's* reproduction of original by Von Boros.†) "To calculate the thickness, the intersection of the diagonal line, representing the measured mean diameter, with the vertical line, representing the measured mean corpuscular volume, is spotted. A line down from this point crosses the graduated vertical line at the left at the mean erythrocyte thickness."—*Reproduced by courtesy of the* American Journal of Laboratory and Clinical Medicine.

the "thickness index" of Von Boros as the "volume thickness index," and presented a nomogram (Figure 2) by which it is easily determined.

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* Haden, Russell L. The volume thickness index of the erythrocyte of man. *J. Lab. Clin. Med.*, 20: 567, 1935. (cf. Ref. 118)

† Von Boros, J. *Die Behandlung der Anämien, Ergeb. d. inn. Med. u. Kinderh.*, 42: 635, 1932.

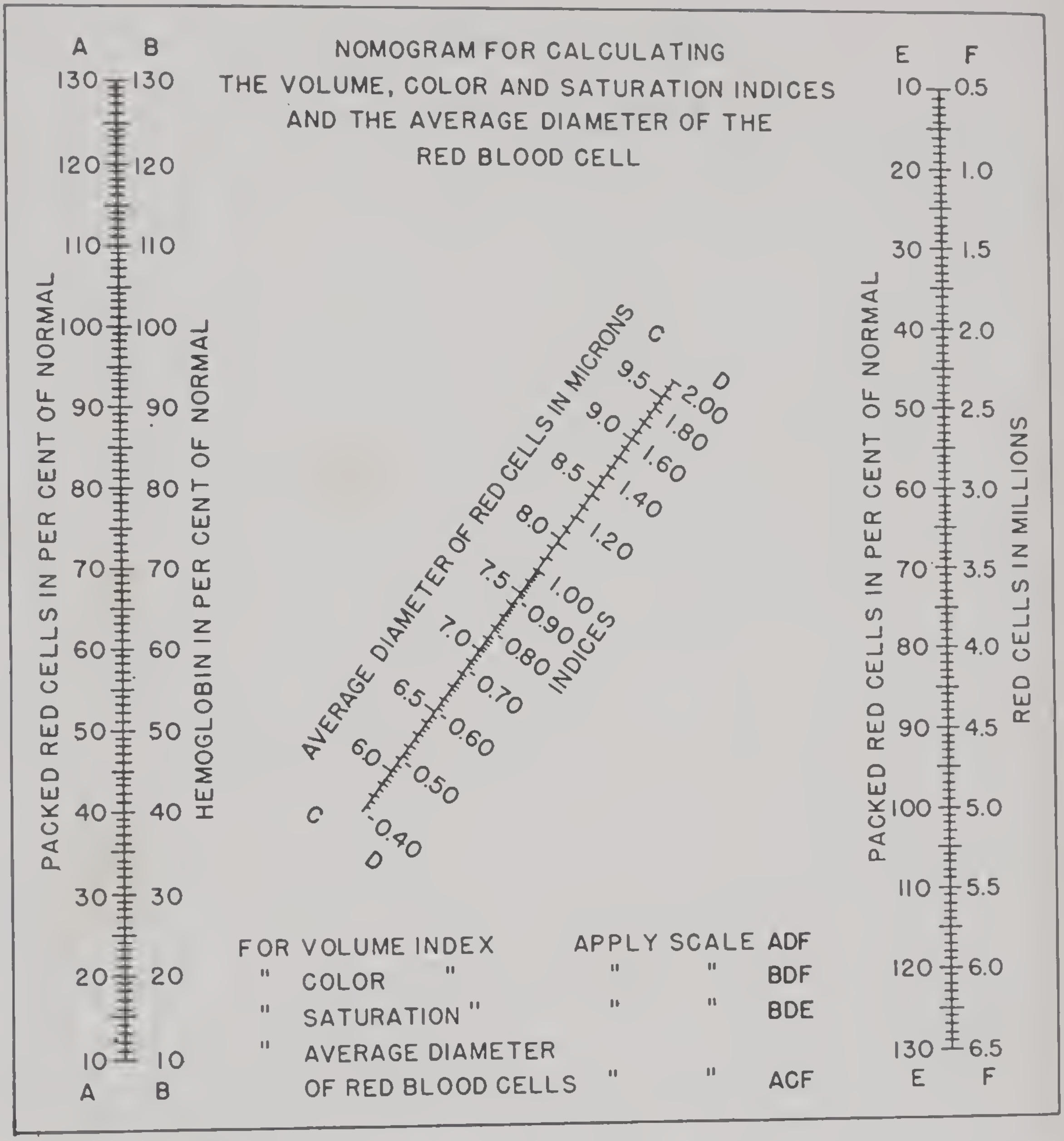
NOMOGRAM FOR CALCULATING THE VOLUME, COLOR AND SATURATION INDICES AND THE AVERAGE DIAMETER OF THE RED BLOOD CELL
A
B
C
D
E
F
PACKED RED CELLS IN PER CENT OF NORMAL
HEMOGLOBIN IN PER CENT OF NORMAL
AVERAGE DIAMETER OF RED CELLS IN MICRONS
INDICES
PACKED RED CELLS IN PER CENT OF NORMAL
RED CELLS IN MILLIONS
130 120 110 100 90 80 70 60 50 40 30 20 10
6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5
0.40 0.50 0.60 0.70 0.80 0.90 1.00 1.20 1.40 1.60 1.80 2.00
10 20 30 40 50 60 70 80 90 100 110 120 130
0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5
FOR VOLUME INDEX APPLY SCALE ADF
" COLOR " " " BDF
" SATURATION " " " BDE
" AVERAGE DIAMETER OF RED BLOOD CELLS " " ACF

Figure 2. Nomogram for calculating the indices of the erythrocyte from the number, the hemoglobin and the hematocrit value of the erythrocyte. The mean diameter can also be calculated from the red cell count and hematocrit reading (after Haden's* adaptation of original by Warburg†): (1) Determine the mean corpuscular volume in cubic microns and the volume index of the unknown blood. (2) Measure the diameter of at least 200 red cells and calculate the mean erythrocyte diameter of the unknown blood. (3) Column D in the nomogram shows the volume index of the erythrocyte corresponding to different diameters. Find the volume index corresponding to the measured mean erythrocyte diameter of the unknown blood. (4) The mean corpuscular volume corresponding to the volume index is calculated by multiplying the normal mean corpuscular volume by the volume index. Calculate the mean corpuscular volume of the unknown blood by multiplying the normal mean corpuscular volume by the volume index found corresponding to the measured mean diameter of the unknown blood. (5) Calculate the volume thickness index (V-T I) by dividing the mean corpuscular volume of the unknown blood, as determined directly from the cell count and hematocrit reading, by the calculated mean corpuscular volume corresponding to the measured mean erythrocyte diameter.—*Reproduced by courtesy of the* American Journal of Laboratory and Clinical Medicine.

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* Haden, Russell L. The volume thickness index of the erythrocyte of man. *J. Lab. Clin. Med.*, 20: 567, 1935. (cf. Ref. 118)

† Warburg, *Acta Med. Scan.*, 66: 187, 1927.

Posthemolytic residue (stroma):* Centrifuge a large sample of defibrinated or citrated blood, preferably several liters, one-half hour at 2000 R.P.M. in 250 ml. centrifuge bottles to separate the cells and plasma. Remove the plasma and the layer of white blood cells by suction. Fill the bottles to within an inch of the top with chilled physiological saline solution (0.85 to 0.9 per cent NaCl) and thoroughly suspend the erythrocytes by vigorous shaking. Separate the erythrocytes by centrifugation (one-half hour at 2000 R.P.M.) and draw off the saline wash solution by suction. Repeat the saline washing procedure two additional times. Measure the washed erythrocytes in a graduated cylinder to determine their approximate volume.

One volume of washed erythrocytes is hemolyzed in 20 volumes of chilled dilute citrate buffer solution.† Adjust to *pH* 5 to 5.5, using a glass electrode, and allow to stand overnight in the refrigerator. Pass the mixture through a Sharples supercentrifuge, 30 to 40 thousand R.P.M. Wash the posthemolytic residue (stroma) in the centrifuge with chilled, dilute buffer and resuspend in one to two liters of chilled, dilute buffer. Rotate the suspension in a ball mill for an hour and pour into 20 volumes of the chilled dilute buffer. Leave for several hours in the refrigerator, then run the suspension through the supercentrifuge and wash as before (carrying through the complete process to resuspension in 20 volumes of chilled dilute buffer) and, after four washings in all (wash solutions should be free of hemoglobin at this stage) scrape the residue material from the centrifuge bowl and dry *in vacuo* under nitrogen, below 50° C. (See p. 219). For lipid analyses, the dried material may be extracted as described for platelets, p. 310.

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* Bernstein, Samuel, Robert L. Jones, Betty Nims Erickson, Harold H. Williams, Ira Avrin, and Icie G. Macy. A method for the preparation of posthemolytic residue or stroma of erythrocytes. *J. Biol. Chem.*, 122: 507, 1938. (cf. Ref. 16)

† Dissolve 21.008 gm. citric acid ($C_6H_8O_7 \cdot H_2O$) in 200 ml. of carbonate-free 1.0 *N* NaOH and dilute to one liter. For hemolysis dilute 1:15 with water and adjust to *pH* 5 to 5.5 with normal citric acid (70 gm./liter) using a glass electrode to measure the *pH*.

CALCIUM DETERMINATION IN BLOOD

Serum, heparinized plasma or oxalated whole blood containing as much as 0.3 per cent potassium oxalate, may be used for the determination of calcium in blood. The method is based upon the procedure given by Peters and Van Slyke.* If sufficient material is available, 5 ml. of serum or plasma yields enough protein-free filtrate for duplicate analyses. If minimal amounts of material must be used, 1 ml. of blood or serum can be ashed, the ash redissolved in dilute hydrochloric or nitric acid, and the ash solution used for analysis.

Procedure

Pipette one volume of serum or plasma into a volumetric flask calibrated to hold five times the volume of the sample. Mix the sample with three volumes of water, followed by one volume of freshly prepared 20 per cent trichloracetic acid,† filling the flask up to the mark. If whole blood is used, add seven volumes of water and two volumes 20 per cent trichloracetic acid to one volume of blood; or, mix directly with one volume of blood, nine volumes of five per cent trichloracetic acid. Mix and allow to stand one-half hour for precipitation of the proteins. Transfer the mixture to a tube and centrifuge. Pour off the supernatant liquid through a small, ashless filter paper and collect the filtrate in a small Erlenmeyer flask.

Place an aliquot of the trichloracetic acid filtrate containing about 0.2 mg. calcium (equivalent to 2 ml. of serum, or 3 to 4 ml. of whole blood) in a scrupulously clean, graduated, 15 ml. centrifuge tube‡ and add water to make 10 ml. This volume must not be exceeded. (With whole blood, an aliquot consisting of 20 ml. of filtrate may be required for the determination and must be evaporated to 10 ml.) Add 1 ml. of 20 per cent sodium acetate,§ six to eight drops 0.016 per cent brom cresol green indicator,* and 1 ml. saturated ammonium oxalate (about four per cent). The oxalate should be dropped directly into the solution. Stir the mixture with a thin-footed, glass rod. Add 1:1 solution of concentrated NH_4OH, dropwise, until the resulting color matches that of a similar volume of acetate buffer solution,† pH 5.0, containing the same amount of indicator. Wash off the stirring rod with a few drops of water, cover the tube and allow to stand over night to complete the precipitation of calcium oxalate.

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* Peters, J. P., and Donald D. Van Slyke. Quantitative Clinical Chemistry, Vol. II, Methods, p. 767. The Williams and Wilkins Co., Baltimore, 1932. (cf. Ref. 263)

† Trichloracetic acid, 20 per cent: Dissolve 20 gm. of trichloracetic acid in water and dilute to 100 ml.

‡ Clean tubes may be kept immersed in cleaning solution until used, or may be heated to about 100° C. in the acid mixture immediately before they are used. (Pour one liter of concentrated H_2SO_4 into 35 ml. of a saturated solution of potassium dichromate.)

§ Sodium acetate, 20 per cent: Dissolve 20 gm. of $NaC_2H_3O_2 \cdot 3H_2O$ in water and dilute to 100 ml.

Centrifuge the precipitate until it is well packed in the bottom of the tube (10 minutes at 1500 R.P.M. are usually sufficient) and wash according to Clark and Collip's decantation method: Carefully pour off the supernatant liquid and, keeping the tube inverted, place in a rack with the mouth of the tube resting on a pad of filter paper. Allow five minutes for drainage. Wipe dry the mouth of the tube with a soft cloth and wash the sides with 3 ml. of ammonia solution containing 2 ml. of concentrated NH_4OH per 100 ml., delivered from a wash bottle in a very fine stream. Stir precipitate and recentrifuge. Decant the supernatant solution and again drain by inverting over filter paper for five minutes. Dissolve the precipitate by blowing 2 ml. of approximately normal sulfuric acid directly onto the material in such a manner as to break it up and facilitate solution. Place the tube in a boiling water bath for one minute; then in a beaker containing water heated to 70–75° C. and titrate with 0.01 N $KMnO_4$,‡ from a microburette graduated at 0.02 ml. intervals, until a definite pink color persists for one minute. One ml. of 0.01 N $KMnO_4$ is equivalent to 0.2 mg. of calcium. A blank determination should be run on reagents.

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* Brom cresol green, 0.016 per cent: Dissolve 0.1 gm. of brom cresol green, and 3.2 ml. of 0.05 N NaOH in water and dilute to 100 ml. Dilute 16 ml. of this stock solution to 100 ml.

† Acetate buffer solution: For pH 5.0 use 70.0 ml., 0.2 N sodium acetate plus 30 ml., 0.2 N acetic acid. Two-tenths normal sodium acetate: Dissolve 27.22 gm. of sodium acetate, $NaC_2H_3O_2 \cdot 3H_2O$, in water and dilute to one liter. Two-tenths normal acetic acid: Approximately 11.3 ml. of 99 per cent glacial acetic acid diluted to one liter with water and standardized against 0.1 N NaOH, with phenolphthalein as indicator.

‡ Potassium permanganate, 0.01 N: See footnote, p. 257.

PHOSPHORUS IN BLOOD

Total phosphorus and total lipid phosphorus, are determined by the same procedure. Total phosphorus is determined upon 0.2 ml. of heparinized whole blood, or 0.5 ml. of plasma or serum. Total lipid phosphorus is determined upon an alcohol-ether extract of serum, plasma or whole blood. The method is based upon the procedure given by Kirk[*] and modified by Hoagland.[†]

Procedure

Pipette 0.2 ml. whole blood, 0.5 ml. of plasma or serum, or an aliquot of alcohol-ether extract containing 0.02 to 0.08 mg. of phosphorus, into a 200×15 ml. pyrex digestion tube[‡] constricted to 10 to 12 mm. in diameter at a 25 ml. calibration mark. (The alcohol-ether extract must be evaporated to dryness in a water or steam bath.) Add 1 ml. of sulfuric-nitric acid digestion mixture[§] and a pyrex glass bead or Hengar quartz crystal. Heat the tube with a microburner, under the hood, until a dark brown color appears. Remove from the flame and, while hot, add more HNO_3,[+] a drop at a time, and continue the digestion. Repeat this process (two or three times) until the liquid is perfectly clear. Continue heating until white SO_3 fumes appear and for the next 10 minutes apply enough heat to keep the tube filled with white fumes, but not enough to drive them out of the tube.

Allow the digest to cool, then add 5 ml. of water and one drop of phenol red solution (0.04 per cent aqueous solution), followed by concentrated NH_4OH, dropwise. As the NH_4OH is added the solution acquires the usual yellow color of phenol red in dilute acid. When an excess has been added, the alkaline red color of the indicator appears. Add 1 *N* HCl, drop by drop, until the red color just disappears. Cool contents of the tube and make volume to 25 ml. with water. Mix thoroughly and pipette a 6 ml. sample into a 15 ml. combustion-centrifuge tube.

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[*] Kirk, Esben. Gasometric microdetermination of phosphoric acid. *J. Biol. Chem.*, 106: 191, 1934. (cf. Ref.180)

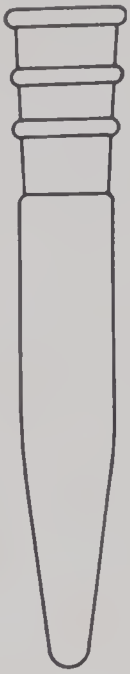
[†] Hoagland, Charles L. Microdetermination of sulfate and phosphate by manometric combustion of their organic precipitates. *J. Biol. Chem.*, 136: 543, 1940. (cf. Ref. 140)

[‡] Scientific Glass Apparatus Co., Bloomfield, N. J., Cat. #M-2525a.

[§] Sulfuric-nitric acid digestion mixture: Equal volumes of highest purity, concentrated, sulfuric and nitric acids free of phosphoric acid.

[+] Further addition of sulfuric acid is unnecessary and should be avoided, to prevent too great concentration of ammonium sulfate in the diluted and neutralized digest. Concentrated solutions of ammonium sulfate may, in themselves, cause precipitation when strychnine molybdate reagent is added.

Add, rapidly 2 ml. of strychnine molybdate reagent* and mix contents of the tube. (Mixing is best accomplished by rotating the tube around its axis while the tube is held in an oblique position.) Set the tube aside for 20 to 45 minutes, and repeat the mixing process once or twice during the interval. If the contents are mixed as described, a flocculent precipitate forms within a few minutes and quickly settles to the bottom of the tube. Twenty minutes suffice for complete precipitation of the phosphorus as strychnine phosphomolybdate. If the mixture stands longer than one hour slight resolution of the precipitate may occur. With each series a blank is run, using all the reagents but substituting distilled water for the sample. The milligrams of phosphorus in the precipitate are found by dividing its carbon content in milligrams by 28.4.



Combustion centrifuge tube, 15 ml., specially prepared by Corning Glass Works, Corning, N. Y.

Centrifuge five minutes at 2000 R.P.M.; decant all the supernatant fluid; allow the tube to drain 10 minutes, inverted over filter paper, in a rack. Wash precipitate three times, each time adding 2 ml. of one per cent HNO_3, and centrifuging for five minutes at 2000 R.P.M. Stir the precipitate in the first two washings. Decant the supernatant liquid after each washing.†

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* Strychnine molybdate reagent:

Solution *A*. Dissolve 50 gm. of ammonium molybdate in 150 ml. of warm water. If not clear the solution should be filtered.

Solution *B*. Two volumes of concentrated HNO_3 and one volume of water.

Solution *C*. Pour 1 volume of solution *A* into three volumes of Solution *B*.

Solution *D*. Strychnine nitrate, 7.5 gm., in water to make 500 ml. of solution. The water may be warmed to facilitate solution.

One volume of solution *D* is poured into three volumes of solution *C*. This constitutes the strychnine molybdate reagent. The reagent should stand at least 24 hours (frequently two weeks are necessary) before it is used. It will keep several months. After the reagent has stood, a slight precipitate forms which should be filtered off. The reagent, when used, should be absolutely clear; it is convenient to filter a small portion of the stock solution immediately before use. Two milliliters of the reagent will precipitate 0.2 mg. of phosphorus.

† Recently, we have substituted a spectrophotometer procedure for the Van Slyke combustion determination. After washing, dissolve the strychnine phosphomolybdate in 2 ml. of 1 per cent NaOH with the aid of a stirring rod. Add approximately 8 ml. of water and transfer to a 100 ml. volumetric flask. Wash

After the last washing and drainage the material is ready for combustion and determination of carbon by the Van Slyke manometric procedure (p. 285).

Inorganic phosphorus: Pipette 1 ml. of serum, plasma or heparinized whole blood into a 10 ml. volumetric flask containing 5 ml., 10 per cent trichloracetic acid* and fill the flask to the mark with water. After 15 minutes filter through ash-free filter paper. In analyses of normal blood or plasma, 3 ml. of the filtrate are used for each determination. In analyses of whole blood or cells, the usual precautions for preventing hydrolysis of acid-soluble organic phosphorus compounds must be observed.†

Place the aliquot of the filtrate in a 15 ml. combustion-centrifuge tube, calibrated at 6 ml., and add one drop of 0.04 per cent phenol red indicator solution, followed by one drop of concentrated NH_4OH. The color of the solution changes from yellow to red. Add 1 *N* HCl, dropwise, until the color again changes to yellow, then add water to make 6 ml. Precipitation and determination of phosphorus are carried out as described for total phosphorus.

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the tube twice with 10 ml. portions of water, adding the wash solutions to the flask. Fill to volume.

Read the per cent transmittance in a Coleman Universal Spectrophotometer, using a PC6 filter for ultraviolet light, at a wave length of 375 mmμ. The reference solution is a blank which has been carried through the procedure. This method has been found accurate within a range of 0.0025 to 0.03 mg. phosphorus, by comparison with duplicate determinations carried out with the Van Slyke procedure.

* The 10 per cent solution of trichloracetic acid should be tested for phosphates, by blank analysis. If any precipitate is obtained with strychnine molybdate, the trichloracetic acid is purified by distillation *in vacuo*.

† If whole blood or cells are to be analyzed for inorganic or acid-soluble phosphorus, the proteins should be precipitated with an acid coagulant, without previous laking of the cells, in order to prevent hydrolysis of organic phosphoric acid esters. The determination of inorganic phosphate in the filtrate must be made by a method which avoids heat, and should be carried out soon after the filtrate is obtained, because allowing the acid solution to stand, even at room temperature, may cause a slow acid hydrolysis of the organic phosphoric esters. When the plasma or serum phosphate is to be determined, the blood should be centrifuged quickly after it is drawn, and hemolysis must be prevented, in order to avoid spontaneous hydrolysis of organic cell phosphorus into inorganic. There is little, if any, acid-soluble organic phosphorus in the plasma, therefore, after the proteins have been precipitated, the plasma filtrate may even be heated with dilute sulfuric acid without significantly increasing the inorganic phosphate content.

CHLORINE*

Serum, plasma or heparinized whole blood may be used for the determination of chlorine in blood. The method is based upon the procedure given by Peters and Van Slyke.†

Procedure

Add to 1 ml. of whole blood, plasma, or serum, in a 25×200 mm. pyrex glass test tube, slowly and with *constant stirring*, 3 ml. of 0.05 N silver nitrate in concentrated nitric acid.‡ Cover the tube with a watch glass and immerse the lower part in a steam bath until the solution above the silver chloride precipitate is clear and light yellow in color. (One to two hours suffice for serum; with whole blood 12 hours are required.) A few drops of a saturated solution of potassium permanganate may be added to diminish the color of solutions from whole blood. Digests of plasma or serum are of such light color that no appreciable gain in accuracy of determining the end-point is achieved by using permanganate.

When digestion is complete, cool the tube to room temperature or below; add 6 ml. of five per cent ferric alum; and titrate the excess silver nitrate with 0.02 N thiocyanate§ until a pink color appears and persists 15 seconds. Cooling the contents of the tube before titration is important because the end-point of the titration becomes increasingly sharp as the temperature diminishes. One milliliter of 0.02 N silver nitrate is equivalent to 0.709 mg. of chlorine.

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* Chlorine and chloride have been used interchangeably in referring to Cl in blood.

† Peters, J. P., and Donald D. Van Slyke. Quantitative Clinical Chemistry, Vol. II, Methods, p. 836. Williams and Wilkins Co., Baltimore, 1932. (cf. Ref. 263)

‡ Dissolve 8.495 gm. fused silver nitrate in a minimum amount of water and add concentrated HNO_3 (sp. gr. 1.4) to make one liter of solution. Solution will keep indefinitely.

§ Thiocyanate solution, 0.02 N: Dissolve 1.6 gm. of sodium thiocyanate (NaSCN), 1.5 gm. of ammonium thiocyanate (NH_4SCN), or 1.9 gm. of potassium thiocyanate (KSCN), in about 900 ml. of water. Filter the solution if not clear. Use this solution to titrate 3 ml. of the 0.05 N acid silver nitrate solution, under the conditions described for blood analysis, adding 6 ml. of 5 per cent ferric alum solution as indicator. From the titration results calculate how much to dilute the thiocyanate so that 7.54 ml. will be required to titrate 3 ml. of the 0.05 N acid silver nitrate solution. (The extra 0.04 ml. is the excess of thiocyanate required to give the end-point.) Dilute the stock solution of thiocyanate to the calculated volume, and check by repeating the titration. The titration value of the thiocyanate should be redetermined at least once every two weeks.

POTASSIUM IN BLOOD

Serum, plasma or heparinized whole blood may be used for the determination of potassium. The method is based upon the procedure given in Peters and Van Slyke.[*] There is not much latitude in the procedure and the amounts determined *must* range between 0.2 and 0.5 mg. potassium. The amount of potassium in 1 ml. of plasma or serum (or the ash[†]), or in 0.1 to 0.2 ml. of whole blood,[‡] is usually within this range.

Procedure

Measure a sample containing 0.2 to 0.5 mg. potassium into a graduated 15 ml. pyrex centrifuge tube; add 0.5 ml. of sodium nitrite solution[§]; mix thoroughly, and allow to stand five minutes. Add water to make the volume 4 ml.; mix the contents and continue the analysis as described for urine (p. 261).

SODIUM IN BLOOD

Serum, plasma or heparinized whole blood may be used for determination of sodium. The method is based upon the procedure given by Peters and Van Slyke.[‖]

Procedure

Pipette 1 ml. serum, plasma, or whole blood, into a thick walled pyrex test tube (25×200 mm.). Add a pyrex glass bead (or Hengar crystal), 1 ml. 4 *N* H_2SO_4 and 0.5 ml. concentrated HNO_3. Digest over a microburner under a hood. When charring occurs, remove the flame and carefully add a few drops of 30 per cent hydrogen peroxide,[#] or concentrated HNO_3, letting the liquid run down the side of the tube. Digest again. (If the solution does not clear, repeat the process of digestion, after adding more H_2O_2 or HNO_3. With whole blood, additional H_2SO_4 also may be required to complete the digestion.) Continue heating for a few minutes after the solution has cleared. Cool; add four to five drops of water; then add approximately 15 ml. of uranyl zinc acetate reagent,[+] with stirring. Continue stirring for a few minutes after precipitation occurs. Allow the preparation to stand one hour, then continue as for urine (p. 264). One gram of sodium uranyl zinc acetate equals 14.95 mg. of sodium.

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[*] Peters, J. P., and Donald D. Van Slyke. Quantitative Clinical Chemistry, Vol. II, Methods, p. 748. Williams and Wilkins Co., Baltimore, 1932. (cf. Ref. 263)

[†] The ash should be dissolved in not more than 1 ml. water and may contain free mineral acid equivalent to not more than 1 ml. of 0.1 *N* solution.

[‡] Place 1 ml. whole blood and 3 ml. water in a calibrated, 10 ml. test tube. Add 2 ml., 20 per cent trichloracetic acid; let stand 10 minutes; make to 10 ml. volume and filter. Place 2 ml. of the filtrate in a 15 ml. centrifuge tube and evaporate to dryness. Take up with 1 ml. 0.1 *N* HCl and proceed as for serum.

[§] Sodium nitrite solution: Dissolve 50 gm., Merck's reagent potassium free sodium nitrite in 100 ml. distilled water.

[‖] Peters, J. P., and Donald D. Van Slyke, Quantitative Clinical Chemistry, Vol. II, Methods, p. 736. The Williams and Wilkins Co., Baltimore, 1932. (cf. Ref. 263)

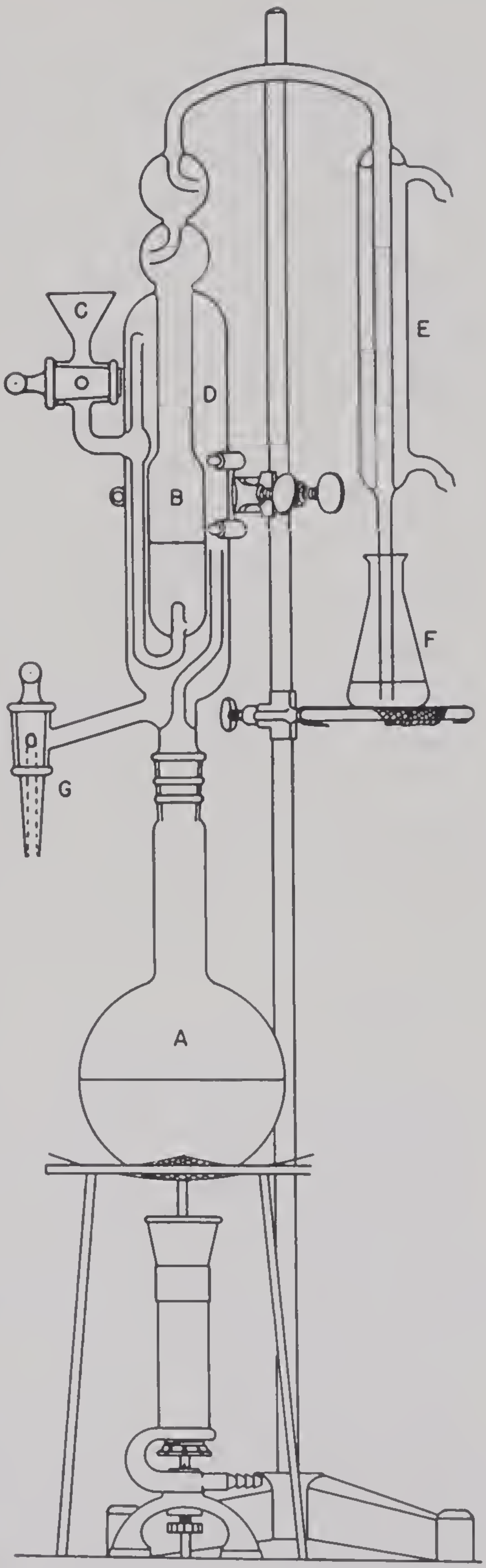
[#] Superoxol.

[+] Uranyl zinc acetate reagent: See footnote, p. 264.

MICRO NITROGEN DETERMINATION

Digest a sample containing 0.5 to 1.5 mg. of nitrogen in a 30 ml. micro Kjeldahl flask with one to two milliliters concentrated H_2SO_4 and one or two small crystals of copper sulphate. (If nitrogen is determined in lipid material dissolved in alcohol-ether mixture, the solvent must be evaporated off before the digestion.) Digestion is obtained by boiling the mixture. Continue boiling five minutes after the solution clears.

After digestion, transfer the solution to the micro distilling apparatus; add 15 ml. of 50 per cent NaOH and steam distill the ammonia into 10 ml. of four per cent boric acid containing 10 drops of methyl red-methylene blue indicator.* Titrate the ammonia with 0.01 *N* HCl. A blank on the reagents run at the same time serves as an end point color standard in titrating the sample. One milligram of 0.01 *N* HCl is equivalent to 0.14 mg. of nitrogen.



Micro Nitrogen Apparatus. A. Steam generator flask. B. Steam distilling chamber. C. Inlet funnel for sample and alkali. D. Outer steam jacket. E. Condenser. F. Flask to trap ammonia. G. Clean-out stopcock.

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[*] Dissolve 500 mg. methylene blue in 100 ml. 95 per cent ethyl alcohol. Dissolve 500 mg. methyl red in 100 ml. 95 per cent ethyl alcohol. Add one part methylene blue to 15 parts of methyl red solution and dilute 1:10 with 50 per cent ethyl alcohol to make the indicator solution.

CEREBROSIDES

In determining cerebrosides a modification[*] of the method given by Kirk[†] has been employed in this laboratory. Recently, Brand and Sperry[‡] have presented an additional modification of the method with the statement:

> Presumably because of an experience similar to ours, Erickson *et al.*[*] intensified Kirk's conditions for hydrolysis; the concentration of hydrochloric acid was increased from 3 *N* to 6 *N*, the time of heating in boiling water was increased from 10 to 15 minutes, and tightly stoppered cylinders instead of open (funnel at top) tubes were used. With this procedure 97 and 98 per cent recovery was obtained in two samples of cerebrosides, but the recovery of cerebrosides added to brain extracts was 10 and 15 per cent too low in two determinations. Furthermore values 10 to 15 per cent too low were usually observed when galactose was carried through this procedure.

The possibility that values obtained by the methods published by Kirk[†] and by this laboratory[*] are too low should be considered in using the data published prior to 1942.

The following method for determination of cerebrosides is excerpted from the paper by Brand and Sperry[‡] and is reproduced by the courtesy of the authors and *The Journal of Biological Chemistry*. Some modifications of the method which have been useful in this laboratory are noted following the procedure.

Experimental

Reagents—

6 *N* hydrochloric acid.

Approximately 6 *N* sodium hydroxide (carbon dioxide-free). The titer should be slightly higher than 6 *N*.

Approximately 1 *N* hydrochloric acid.

0.1 *N* sodium hydroxide.

0.1 per cent chlorophenol red in water. A few drops of 0.1 *N* sodium hydroxide are necessary to dissolve the indicator.

0.1 per cent alkyl sulfate[§] solution.

4.5 per cent zinc sulfate ($ZnSO_4 \cdot 7H_2O$).

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[*] Erickson, Betty Nims, Helen J. Souders, Marion L. Shepherd, D. Maxwell Teague and Harold H. Williams. Relation between cerebroside and neutral fat contents of blood plasma, erythrocytes and stroma. *Proc. Soc. Exp. Biol. Med.*, 45: 153, 1940. (cf. Ref. 86)

[†] Kirk, Esben. A study on Kimmelstiel's procedure for titrimetric cerebroside determination, with description of an improved technique. *J. Biol. Chem.*, 123: 613, 1938. (cf. Ref. 182)

[‡] Brand, Florence C., and Warren M. Sperry. The determination of cerebrosides. *J. Biol. Chem.*, 141: 545, 1941. (cf. Ref. 34)

[§] The "purified sodium alkyl sulfate" was supplied by The Procter and Gamble Company, Chemical Division, Ivorydale, Ohio.

Alkaline ferricyanide solution.[*]

Approximately 18 *N* sulfuric acid.[*]

Stock solution of ceric sulfate, prepared according to the method of Miller and Van Slyke[*] except that it is made 0.15 *N*.

0.003 *N* ceric sulfate solution. 2 ml. of stock solution and 5 ml. of 18 *N* sulfuric acid are diluted to 100 ml.

0.1 per cent solution of alkali-fast erio green.

Washed filter paper, 7 cm. Either Whatman No. 41 or Seavy's No. 106[†] paper is extracted in a continuous extractor[‡] in a Pyrex percolator with water until the blank becomes constant (usually 35 to 40 hours).

Method

(*a*) *Hydrolysis*—Equal portions of a lipid extract containing not over 2.8 mg.[§] of cerebrosides are pipetted into two 10 ml. volumetric flasks, with care that the entire sample is at the bottom of the flask. After removal of the solvent 2 ml. of alkyl sulfate solution are added to each flask, and also to two other flasks to be carried through the procedure as blanks. The flasks are stoppered and placed in boiling water for 5 to 10 minutes, with occasional swirling to emulsify the lipids. To one of the flasks containing lipids and to one of the blank flasks are added 2 ml. of 6 *N* hydrochloric acid; 2 ml. of water are added to each of the others. The flasks are swirled to mix the contents, stoppered tightly, and heated in boiling water for 45 minutes.

(*b*) *Neutralization*—After cooling, one drop of the chlorophenol red[+] solution and 2 ml. of 6 *N* sodium hydroxide are added to the flasks containing hydrochloric acid. One *N* hydrochloric acid is added dropwise until the solution is acid (yellow) and the color is finally brought just to a definite purple by careful addition of 0.1 *N* sodium hydroxide. Two ml. of water are added to each of the other flasks to make the volumes approximately the same in all.

(*c*) *Clarification*—After cooling, 0.5 ml. of the zinc sulfate solution is added to each flask followed by 2 ml. of 0.1 *N* sodium hydroxide. The samples are shaken vigorously. From this point either of two procedures may be used. (*a*) Water is added to the mark and the suspension is mixed and filtered through washed paper into a small flask or test-tube. An aliquot (7 or 8 ml.) is pipetted into a special test-tube[*] or 50 ml. Erlenmeyer flask and water is added to make the total volume about 18 ml. (*b*) The solution is transferred quantitatively through a washed filter into a test-tube[*] or 50 ml. Erlenmeyer flask (four washings with 3 ml. portions of water—total volume about 18 ml.). With this procedure it is not necessary to use volumetric flasks for hydrolysis.

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[*] Miller, B. F., and Van Slyke, D. D., *J. Biol. Chem.*, 114: 583, 1936.

[†] This inexpensive, American made paper may be obtained from M. J. Seavy, 30 Church Street, New York.

[‡] Sperry, W. M. *J. Biol. Chem.*, 68: 357, 1926.

[§] Larger amounts may be analyzed by increasing the quantity of ferricyanide at step (*d*).

[+] Phenol red[182] cannot be used, because it obscures the end-point in the titration with ceric sulfate.

The filtrates from unhydrolyzed lipid samples, *i.e.* those heated without hydrochloric acid, are usually opalescent or slightly cloudy. They may be clarified somewhat by refiltration through the same paper, but this is unnecessary, unless the cloudiness is marked, because it does not affect the result except in so far as it tends to obscure the end-point in titration with ceric sulfate.

(*d*) *Oxidation with Ferricyanide*—Two ml. of the ferricyanide solution are added to each of the tubes or flasks which are fastened in a rack and heated in boiling water according to the directions of Miller and Van Slyke.[*]

(*e*) *Titration*—The titration with ceric sulfate is carried out as described[*] except that alkali-fast erio green is used as an indicator instead of setopaline C, which is not available at present.[†]

Calculation

The factor for galactose was constant over the range from 0.1 to 0.6 mg. The average of thirty-three determinations was 0.147 ±0.002 (S.D.) mg. of galactose per 1 ml. of 0.003 N ceric sulfate. The quantity of cerebroside is calculated from the following equation, 4.55[‡]$\times 0.147((A - B) - (C - D)) =$ mg. of cerebroside, in which A, B, C, and D are the volumes of 0.003 N ceric sulfate solution required to titrate the hydrolyzed lipid sample, hydrolyzed blank, unhydrolyzed lipid sample, and unhydrolyzed blank respectively.

[The method as followed in this laboratory involves the use of

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[*] Miller, B. F., and D. D. Van Slyke, *J. Biol. Chem.*, 114: 583, 1936.

[†] Although alkali-fast green is less sensitive than setopaline C, we obtained equally good results with the two indicators.

[‡] This factor is used on the assumption that approximately equal quantities of phrenosin and kerasin are present. The factor for phrenosin is 4.6 and for kerasin it is 4.5.

10 ml. graduated cylinders instead of 10 ml. volumetric flasks; the substitution of 2 drops of the indicator, 0.025 *M* o-phenanthroline ferrous complex (G. Frederick Smith Chemical Co., Columbus, Ohio), for alkali-fast erio-green; and, the addition of 2 ml. of saturated NaCl to the unhydrolyzed samples, in place of water, just before the addition of zinc sulfate. The NaCl helps to clarify an opalescence which interferes with detection of the end-point.]

CHOLINE*

Add 10 ml. of ethyl alcohol and 5 ml. of saturated barium hydroxide to aliquots (equivalent to 0.15 to 0.5 mg. choline) of the alcohol-ether extract in titer tubes. Heat the tubes on the steam bath until the contents are reduced to a volume of approximately 1 ml.; wash down the sides of the tubes with 10 ml. of ethyl alcohol and reheat until the volume is reduced to 0.5 to 1 ml. Caution must be exercised to prevent them from becoming completely dry. Make the residue slightly acid with 10 per cent hydrochloric acid, using phenol red† as indicator, and filter through a small funnel (3 cm., No. 1, Whatman paper) into a 50 ml. conical centrifuge tube. Wash the tube thoroughly with several 1 ml. portions of water and filter into the centrifuge tube; the final volume should not exceed 10 to 15 ml.

Chill the centrifuge tubes, containing the hydrolysates, to 5 to 10° C. in an ice-salt bath and then add 0.4 ml. of iodine-potassium iodide reagent‡ for each milliliter of solution. After addition of the reagent, allow the tubes to stand in the ice bath for 30 minutes, then centrifuge at 2000 R.P.M. for 15 minutes, and replace in the ice-salt bath. While the tubes are kept cold in an ice-water bath remove the supernatant fluid and wash solutions with Corning fritted glass immersion filter sticks, *M* porosity, which enable washing the labile precipitate with minimum losses from volatilization. Wash the precipitate with 1 ml. portions of water (5 to 10° C.) until the wash solution is colorless (usually five washings suffice), with caution to avoid disturbing or sucking the precipitate onto the filter. The filter stick is allowed to remain in the tube while 5 ml. of bromine solution* are added. Stir up the precipitate with the filter stick and allow the tubes to stand for several hours for complete conversion of the iodide to iodate. Wash the filter sticks with water (force water through the filter to rinse the inside) and remove.

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* Erickson, Betty Nims, Ira Avrin, D. Maxwell Teague, and Harold H. Williams. Micromethods for the determination of sphingomyelin and choline. Applications for the estimation of the phospholipid partition (sphingomyelin, lecithin, and cephalin) in blood and tissues. *J. Biol. Chem.*, 135: 671, 1940. (cf. Ref. 84)

† Phenol red, aqueous, 0.4 per cent.

‡ Iodine-potassium iodide reagent: 15.7 gm. iodine and 20 gm. potassium iodide in 100 ml. water. This solution should be filtered each time before using.

Add 2 ml. of a 25 per cent sodium acetate solution and 10 drops of 90 per cent formic acid. (This should decolorize the solution completely. If a yellow color persists, indicating insufficient bromine to complete the oxidation of the iodine, add more bromine solution and again allow the solution to stand.) Add 5 ml. of freshly prepared 10 per cent potassium iodide solution and titrate immediately with standard 0.005 *N* sodium thiosulfate,† using soluble starch indicator† to determine the endpoint. A blank containing all the reagents should be carried through simultaneously.

Calculation: Choline in sample (mg.) = milliliters 0.005 *N* sodium thiosulfate × 0.01122.

NEUTRAL FAT GLYCEROL

The method of determining neutral fat through its glycerol content may be applied not only to blood lipid extract, but also to extracts of foods, feces and tissues. The procedure, with few modifications, is that outlined by Voris, Ellis and Maynard.‡

Procedure

Evaporate an aliquot of the alcohol-ether extract (containing 0.5 to 2.0 mg. of glycerol combined in neutral fat) to dryness, in a beaker, under nitrogen. Extract residue with 10 to 15 ml. of acetone, warming gently on steam bath; then decant the acetone into a 50 ml. centrifuge tube. Repeat the acetone extraction several times. To the combined acetone extract add 5 drops of a saturated solution of $MgCl_2$ in 95 per cent alcohol to completely remove the dissolved phospholipids: stir vigorously and let stand one hour or longer and centrifuge. Decant the supernatant acetone solution into a 125 ml. Erlenmeyer flask; wash the phospholipid precipitate with 25 to 30 ml. of acetone; recentrifuge, and add the acetone washing to the original acetone solution. Evaporate to dryness under nitrogen; take up residue in 15 to 20 ml. ethyl alcohol; add seven drops of saturated KOH and saponify by heating on steam bath until a pasty mass remains (not less than 30 minutes).

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* Bromine solution: 10 per cent potassium acetate in glacial acetic acid plus 1 ml. of bromine per 100 ml.

† See p. 255.

‡ Voris, L., G. Ellis, and L. A. Maynard. The determination of neutral fat glycerol in blood with periodate. *J. Biol. Chem.*, 133: 491, 1940. (cf. Ref. 363)

Add 1 ml. 10 N H_2SO_4 to hydrolyze the saponified material. Remove the lipid material by extraction with petroleum ether and drive off the last traces of petroleum ether on steam bath. Wash down the sides of the flask with not more than 5 ml. of water and place solution in the refrigerator for one hour; then filter through a fritted glass (medium porosity) Buchner funnel.* The cooling facilitates filtration, which is most readily accomplished by suction, using a test tube within the suction flask as a receiver. The residue is washed several times with water.

For the determination of glycerol, pipette 10 ml. of the periodate reagent† into the water solution of glycerol and allow 20 to 30 minutes for the reaction to become complete. Add 2 drops of 1 per cent p-nitrophenol in 50 per cent alcohol; then neutralize the reaction mixture by introducing dilute NaOH, until a bright yellow color appears, after which add 0.1 N H_2SO_4 dropwise until colorless. Add to the solution, 10 ml. of phosphate buffer‡ followed by 2 ml. of five per cent potassium iodide solution, and titrate the liberated iodine with 0.005 N sodium thiosulfate solution,§ adding starch as indicator, near the end point of the titration. The difference between this titration value and that of a corresponding blank containing all the reagents, represents the sodium thiosulfate equivalent of the glycerol oxidized by the potassium periodate.

Calculation: One milliliter of 0.005 N thiosulfate corresponds to 0.575 mg. KIO_4 or 0.115 mg. of glycerol. The factor 9.62 is used to convert glycerol to triglyceride (neutral fat).

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* Corning Glass Works.

† Periodate reagent: Dissolve 0.6250 gm. KIO_4 in 500 ml. 0.1 N H_2SO_4.

‡ Phosphate buffer: Dissolve 12 gm. $Na_2HPO_4 \cdot 12H_2O$ in water; add 3 ml. 10 N H_2SO_4 and dilute to 100 ml. to obtain *pH* of approximately 5.7.

§ Sodium thiosulfate, 0.005 N: Accurately dilute 50 ml. of standard 0.1 N sodium thiosulfate (see p. 255) to 1000 ml. with carbon dioxide-free distilled water.

SPHINGOMYELIN*

Measure aliquots of the alcohol-ether extract (containing 0.5 to 1 mg. of sphingomyelin) into titer tubes (test tubes 1×4 inches) and evaporate gently on a water bath, with caution to avoid heating or standing after the samples become dry. Dissolve residue in 1 ml. absolute methyl alcohol. Add 1 ml. of an acidified, saturated solution of Reinecke salt in methyl alcohol.† Stopper tubes and chill in refrigerator over night.

Place the tubes containing the sphingomyelin reineckate, which has precipitated during the night, in an ice bath and wash with six 1 ml. portions of ice-cold methyl alcohol, then with six 1 ml. portions of ice-cold ethyl ether, finally with six 1 ml. portions of acetone.[344] Remove the supernatant fluid and washings with a Corning fritted glass immersion filter stick, *M* porosity.

Dissolve the precipitate in four 5 ml. portions of absolute methyl alcohol; heat to boiling (by immersion of the tube in a beaker of boiling water), and then draw off through the filter stick into a phosphorus digestion tube with a 25ml. graduation in the constricted area. Three washings are made with 5 ml. portions of the solvent mixture, which are heated in the titer tube and drawn off into the digestion tube. Evaporate the solution to dryness on a water bath. Carry out digestion, precipitation and gasometric determination of the phosphorus according to the method for total phosphorus (p. 266). A blank determination using an amount of alcohol-ether mixture equal in volume to the size of sample is carried all the way through the procedure.

Calculation: Phosphorus content $\times 25 =$ sphingomyelin content of the sample.

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* Erickson, Betty Nims, Ira Avrin, D. Maxwell Teague, and Harold H. Williams. Micromethods for the determination of sphingomyelin and choline. Applications for the estimation of the phospholipid partition (sphingomyelin, lecithin, and cephalin) in blood and tissues. *J. Biol. Chem.*, 135: 671, 1940. (cf. Ref. 84)

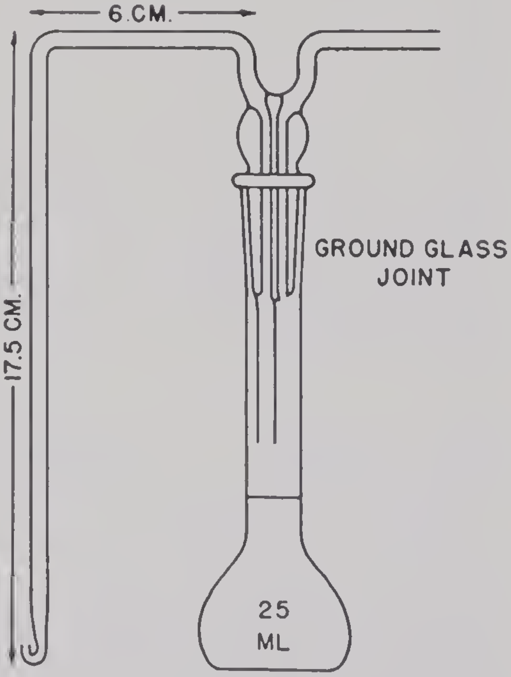
† To 10 ml. of absolute methyl alcohol in a beaker add an excess of Reinecke salt‡ (2–2.5 gm.) and stir vigorously at room temperature. Add 0.16 milliliter of concentrated HCl and let stand 10 to 15 minutes with intermittent stirring, being sure an excess of Reinecke salt is present. (The solution should not be heated as heat decomposes the Reinecke salt.) The solution is filtered and is then ready for use. It should be made up fresh each time as it decomposes with standing.

‡ Eimer and Amend or Eastman Kodak.

CHOLESTEROL*

Manometric Method

Pipette an aliquot (equivalent to 0.15 to 0.5 mg. of total cholesterol) of alcohol-ether extract into a pyrex test tube (17.5 by 22 mm.) and add 2 ml. of a saturated solution of NaOH in redistilled 95 per cent alcohol. Place the test tube in a water bath or an electric oven at 85° C. When the contents of the tube have been reduced to about 3 ml., insert a cork stopper covered with tin foil firmly into the opening of the tube. (The stopper is provided with a small furrow to allow escape of alcohol vapors. The exact control of cholesterol ester saponification requires more care than any other step of the analysis. If treatment with alkali is not severe enough, likely if the temperature during saponification falls much below 85° C., saponification is incomplete and the total cholesterol yield is low. If the treatment is too severe, some cholesterol is destroyed, also producing low results. The latter may occur if the volume of the mixture becomes too greatly reduced towards the end of the saponification.) After two and one-half hours at 85° C., cool the tube; add two drops of 0.04 per cent aqueous phenol red solution; and neutralize contents of the tube with 1 *N* HCl.



From the saponified solution, extract the total cholesterol with five, successive, 5 ml. portions of petroleum ether, and transfer to a 25 ml. volumetric flask. With each portion of petroleum ether, carefully heat the contents of the tube by immersing in a beaker of hot water, and agitate to insure thorough mixing. (Use a heavy stirring rod with a foot or flattened end.) Part of the petroleum ether evaporates; the rest is drawn as completely as possible into a 25 ml. flask through a suction transfer tube. Care must be taken to prevent drops of the water-alcohol solution (lower layer) from being drawn over into the volumetric flask. So much petroleum ether evaporates that the five portions do not fill the 25 ml. flask. After rinsing the ground stem of the capillary of the suction transfer tube, with petroleum ether, make the contents of the flask 25 ml. with petroleum ether. Insert a clean, ground-glass stopper into the flask and mix the petroleum ether solution. Pipette a 10 ml. sample of the petroleum ether solution into a 15 ml. combustion-centrifuge tube (see p. 317) for digitonin precipitation. Evaporate the petroleum ether (gently, under 60° C.) and take up in 1 ml. of ethyl alcohol. Add 0.2 ml. of one per cent digitonin solution (in 50 per cent alcohol) and evaporate the contents of the tube to dryness in a beaker on top of a constant temperature (85° C.) bath. (The contents of the tube remain at approximately 60° C.)

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* Kirk, Esben, Irvine H. Page, and Donald D. Van Slyke. Gasometric microdetermination of lidips in plasma, blood cells, and tissues. *J. Biol. Chem.*, 106: 203, 1934. (cf. Ref. 181)

When dry, wash the residue with three, 10 ml. portions of *anhydrous* ethyl ether, and centrifuge for 10 minutes between each washing. Draw the wash solution off through a fine capillary tube by gentle suction without disturbing the precipitate. Following the third washing, evaporate the small amount of ethyl ether remaining, carefully and *completely*, by immersing tube in warm water. Wash the precipitate with three, 10 ml. portions of hot water; centrifuge and remove the supernatant fluid as before. Completely evaporate the remaining water in the tube by heating in the steam bath and carry out combustion of the precipitate as for total carbon on Van Slyke manometric apparatus. (See method, p. 285.) The milligrams of carbon obtained on combustion of cholesterol digitonide may be converted to milligrams of cholesterol by multiplying by 0.3926.

Free cholesterol: Free cholesterol may be determined on an unsaponified lipid sample. Aliquots of the alcohol-ether extract (equivalent to 0.07 to 0.15 mg. of free cholesterol) are pipetted into the 15 ml. combustion-centrifuge tubes. Two-tenths milliliter of one per cent digitonin solution (in 50 per cent ethyl alcohol) is added and the determination carried out as for total cholesterol. Additional ether washings of the cholesterol digitonide precipitate may be necessary to completely remove all of the contaminating phospholipids as they are most likely to be occluded when cholesterol digitonide is precipitated from an unsaponified solution.

The possibility that determination of free cholesterol in plasma lipids by the above procedure may give results 20 per cent too high, is stated by Folch and Van Slyke[103] who propose avoiding this error by precipitating the digitonide in a medium containing water, absolute alcohol and ether in proportions 2:9:9. The precipitate forms quantitatively without taking to dryness when the mixture is kept overnight in an icebox.

CHOLESTEROL

Colorimetric Method

The procedure for cholesterol determination was published by Schoenheimer and Sperry[*] and revised by Sperry.[†] The method is presented through the courtesy of the authors and *The Journal of Biological Chemistry.*

Procedure

Reagents

1. Acetone (redistilled)-absolute alcohol (1:1).
2. Ether (peroxide free).
3. Acetone (redistilled)-ether (peroxide free) (1:2).
4. Digitonin solution. This solution is prepared by dissolving 1 gm. of digitonin in one liter distilled water. The solution is placed in the ice box for at least 24 hours (preferably longer) and then centrifuged to pack the precipitate[‡] which forms. The supernatant solution is filtered and concentrated to approximately 500 ml. This is best done by placing the solution in a weighed one liter flask equipped with inlet and outlet tubes. The mouth of the inlet tube should be kept about 2 cm. above the surface of the solution. A rapid stream of air, filtered through cotton, is blown, or drawn by suction, through the flask, which is immersed in boiling water. The point at which the required amount of water has been removed is determined with sufficient accuracy by weighing the flask with its contents on an ordinary laboratory balance. The concentration requires three to four hours. Should a sedi-

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[*] Schoenheimer, Rudolf, and Warren M. Sperry. A micromethod for the determination of free and combined cholesterol. *J. Biol. Chem.*, 106: 745, 1934. (cf. Ref. 294)

[†] Sperry, Warren M. The determination of cholesterol. *J. Biol. Chem.*, 118: 377, 1937. (cf. Ref. 325*a*)

Sperry, Warren M. A micromethod for the determination of total and free cholesterol. *Am. J. Clin. Path.*, Tech. Suppl., 8 (2): 91, 1938. (cf. Ref. 325*b*.)

We are grateful to Dr. Sperry for revising the galley proof of the cholesterol method so that it contains his most recent modifications of the procedure (March, 1941).

[‡] The nature of this substance, which precipitates only from dilute aqueous solutions, is not known. It forms rapidly in a 0.1 per cent solution but comes down slowly in a 0.2 per cent solution. We have some evidence indicating that it tends to give high values when left in the solution though we have not studied the point extensively. We have employed only Hoffmann-La Roche, Inc., digitonin which we have found to give uniform results. It is possible, in view of the findings of Schoenheimer and Dam, that other samples of digitonin may not behave similarly.

ment appear in the concentrated solution on standing, it should be removed by filtering.

5. Potassium hydroxide solution made by dissolving 10 gm. of pure KOH in 20 ml. of water.

6. Hydrochloric acid, approximately five per cent, made by diluting 15 ml. of concentrated HCl to 100 ml.

7. Acetic acid, glacial. Either Eastman or Baker's C.P. products are recommended.

8. Acetic anhydride. The product, labeled "acetic anhydride 99–100 per cent," supplied by the Eastman Kodak Company has been uniformly satisfactory.[*]

9. Concentrated sulfuric acid.

Special Apparatus Required

1. Volumetric flasks, 5 ml.

2. Funnels, 2.5 cm. in diameter.

3. Filter paper, 4.5 cm. in diameter. The filter paper must be extracted with ether or hot alcohol until completely free of sterols.

4. Stirring rods, approximately 13 cm. long.

5. Preserving jars, either pint or quart sizes, with rubber gaskets.

6. Dropping bottles with ground-in pipettes equipped with rubber bulbs.

7. Centrifuge tubes, pyrex, 15 ml., calibrated at 2 ml.

8. Transfer pipettes, for use with rubber bulbs. These pipettes are made by drawing out 8 mm. glass tubing. The total length should be approximately 13 cm., and the tip should be approximately 5 cm. long. The orifice should be large enough to permit rapid filling and emptying.

9. Water bath, equipped to hold 15 ml., centrifuge tubes in the dark at 25°.[†]

10. Zeis Pulfrich photometer equipped with 5 cm. microcells, a micro-photoelectric photometer, described by Weech,[‡] or any other color-measuring instrument of equivalent accuracy. The

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[*] Kahlbaum's acetic anhydride "pro analysis" gives values approximately five per cent lower than the Eastman preparation. A fraction, boiling at 137° (about two-thirds of the original sample), obtained by fractionation of the Kahlbaum acetic anhydride with an efficient column, gave the same values as the Eastman product.

[†] Any pan of fairly large capacity, fitted with a rack and a thermometer, is suitable. The pan may be placed in a box with a door or simply covered with a dark cloth to exclude light.

[‡] Weech, A. A. A micro-photoelectric photometer. *Proc. Soc. Exper. Biol. Med.*, 45: 858, 1940. (cf. Ref. 372*a*)

color filter or filter pack should have a maximal transmission at as nearly 625 millimicrons as possible and should not transmit much below 600 millimicrons. Zeiss filter No. 61 is used with the Zeiss Pulfrich photometer and Corning filters 244 and 978 with the photoelectric photometer.

Extraction. Approximately 2 ml. of the acetone-absolute alcohol solution are placed in a 5 ml. volumetric flask; 0.2 ml. of serum or whole blood is run in slowly, without shaking during the addition but with vigorous shaking immediately after withdrawal of the pipette. The solvent is brought to a boil (steam bath) with agitation to prevent bumping, and after being cooled to room temperature the contents are made to volume with alcohol-acetone, mixed thoroughly, and filtered through a small, dry filter. The filtrate should be perfectly clear.

The proportion of serum may be varied widely to compensate for expected abnormalities in cholesterol concentration. For example in analyzing normal rabbit serum 0.4 or 0.5 ml. is usually extracted while the ratio of hypercholesterolemic sera to volume of extract is reduced (usually 0.2 or 0.1 ml. of serum is extracted in 10 ml. volumetric flasks). Should duplicate determinations be desired, the amounts may be increased in approximate proportion. Good results have been obtained with extracts made up in 10 ml. flasks from 0.5 ml. of serum.

Precipitation of free cholesterol.* Two ml. of the filtrate are pipetted into a centrifuge tube, 1 ml. of the digitonin solution and 1 drop of 5 per cent hydrochloric acid are added, and the solution is stirred thoroughly with a stirring rod which is left in the tube. The tube is placed in a preserving jar, the cover is placed on tightly, and the jar is left overnight at room temperature. The tube is transferred to a test tube rack and its contents are stirred gently to free particles of precipitate which may adhere to the walls of the tube near the surface of the liquid. The stirring rod is removed carefully without touching the upper part of the tube and laid on a rack made of heavy wire and so designed that a number of rods may be held without danger of rubbing off adherent precipitate. The tube is centrifuged until the precipitate is packed tightly enough to permit decantation of the supernatant liquid without appreciable loss of suspended material. Centrifuging for 15 minutes at 2800–3000 R.P.M. is adequate, but there is some danger of breakage at this speed unless Pyrex tubes are used. If the centrifuge will not run so fast it may be necessary to increase the time. A few small particles usually float at the surface and cannot be centrifuged down. Their loss does not affect the result. After decantation the tube is drained for a few moments and the last drop is removed by touching the lip to a clean towel.

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* The procedure is described for a single determination. In practise the average time per determination may be greatly shortened by carrying through a number of analyses (preferably 16 to 18) together.

The stirring rod is replaced in the tube, and the wall of the tube and the rod are washed down with 1.5 to 2.0 ml. of acetone-ether solution, best added from a dropping pipette with a rubber bulb attached. The precipitate is stirred up thoroughly, the rod is removed to the rack, the tube is centrifuged for 5 minutes, and the supernatant solution is removed as before. The precipitate is washed twice more in the same manner, except that ether instead of acetone-ether is used. The precipitate does not pack so well under ether and it may be necessary to increase the time of centrifuging somewhat to prevent loss at this stage. After the last wash solution has been removed, the stirring rod is returned to the tube and left there through the rest of the procedure. The precipitate becomes dry in a short time at room temperature; if it is desirable to hasten the process the tube may be placed in a water bath at about 40° and the last traces of ether may be removed by a gentle current of air. The sample is now ready for color development. It may be stored for several days at this stage.

Precipitation of total cholesterol: 1 ml. of the extract is pipetted into a 15 ml. centrifuge tube and one drop of the KOH solution is added* and stirred into solution with a stirring rod which is left in the tube. Stirring must be continued until no aqueous solution can be seen at the tip of the tube. If the drop is too large it may be impossible to dissolve it completely.* The tube is placed in a preserving jar containing a layer of sand about 3 cm. deep which has been heated to about 40°. The cover is clamped on tightly and the jar is kept at 37–40° (we utilize an ordinary incubator) for one-half hour.

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* The size of the drop may be varied within wide limits by changing the height of the burette, or pipette tip above the surface of the solution. The closer the tip, the smaller will the drop be as acetone and alcohol vapor lower the surface tension of the alkali solution. A few trials with blank samples will determine the proper position of the tip. The drop should be of such a size that approximately 0.1 ml. of 5 per cent HCl solution are required to neutralize it.

After hydrolysis the tube is cooled, acetone-alcohol solution is added to the 2 ml. mark, and the solution is titrated with 5 per cent HCl (phenolphthalein indicator). The solution is stirred after the addition of each drop, and care is taken to insure that a definite excess of acid is present. Small amounts of HCl have no effect on the precipitation of cholesterol with digitonin, but alkali interferes. One mililliter of digitonin solution is added, the solution is stirred thoroughly (the suspended salt dissolves immediately), the tube is allowed to stand 1 hour or longer, preferably overnight, and the precipitate is centrifuged, washed, and dried in the manner described for free cholesterol except that only one ether washing is necessary.

Development and reading of color. A shallow dish containing a layer of sand about 1 inch deep is heated to 110–115° in an oven. The tubes containing the precipitate are placed in the sand and the dish is returned to the oven for one-half hour. This step is necessary in humid weather during which the hygroscopic digitonide may absorb enough moisture from the air to interfere with the determination. Immediately after removal of the dish from the oven 1 ml. of acetic acid is added while the tube is still in the hot sand. (In case the precipitate appears to be too great to read, the analysis may be saved by adding double amounts of acetic acid, acetic anhydride, and sulfuric acid.) The acid should be so added that any digitonide which may have adhered to the walls of the tube will be washed down. The contents of the tube are stirred vigorously for a few moments and the tube is removed from the sand. Solution occurs immediately except for a few particles of unknown composition which sometimes resist solution even with long heating, especially in the free cholesterol determination. They settle rapidly and do not affect the determination if care is taken not to draw them up in the pipette when the sample is transferred to the photometer or colorimeter cup.

Next, the temperature of the water bath is adjusted to 25°, and the tubes* are placed in the bath and left for a few moments to bring them to temperature equilibrium. One is removed, placed in a rack in a small pan, or beaker, containing water at 25°. Two ml. of acetic anhydride are added, followed by 0.1 ml. of concentrated H_2SO_4 best added from an automatic microburette.* The solution is now stirred vigorously (the original stirring rod is still in the tube), and the tube is replaced in the water bath. Another tube is removed to the small bath and the reagents added as before. The interval between the addition of reagents is so timed that not less than 27 minutes nor more than 37 minutes elapse between the addition of H_2SO_4 and reading. With practise about two minutes are required for a reading; it is possible, therefore, to carry through 16 to 18 determinations in a series. The temperature of the bath is kept at 25° during the procedure.

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* The procedure of color development and reading is described for a series of determinations (see footnote p. 333).

In reading the color with the Pulfrich photometer one of the cells is filled with a blank solution (1 cc. of acetic acid, 2 cc. of acetic anhydride, and 0.1 cc. of H_2SO_4); the other cell is filled with the unknown colored solution, a transfer pipette being used.† The percentage transmission is read, the cells are reversed, and the reading is taken on the opposite side of the instrument (see manufacturer's directions for details). The solution is poured out, and the cell is washed out twice with approximately 0.5 ml. portions of the next unknown solution, a clean transfer pipette being used. The percentage transmission is read as before.

TABLE I

SPECIFIC EXTINCTION COEFFICIENTS OF DIFFERENT AMOUNTS OF CHOLESTEROL PRECIPITATED ACCORDING TO PROCEDURE DESCRIBED

Amount	No. of determinations	Average $E_{sp.}$	Standard deviation	Probable error*
mg.				
0.15	15	1.447	±0.0111	±0.0019
0.10	18	1.454	±0.0110	±0.0017
0.05	20	1.449	±0.0226	±0.0034
0.04	11	1.454	±0.0115	±0.0023
0.03	12	1.447	±0.0226	±0.0044
Average....		1.450	±0.0174	±0.0013

* With 0.025 mg. the probable error was found to be somewhat larger than the values shown. Very little color is produced by such small amounts, and the error in reading is correspondingly great. Amounts smaller than 0.03 mg. are rarely met with in working with serum or blood.

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* The measurement of 0.1 cc. of H_2SO_4 is difficult since drainage is slow. We have found it best to measure the acid by counting drops. Four drops from our burette are equivalent to almost exactly 0.1 ml.

† Occasionally an insoluble sediment of unknown nature is present. It settles during the development of color and does not interfere with the reading if care is taken not to stir it up when withdrawing solution for filling the cell.

If the photoelectric photometer* is used, the cell is filled with a blank solution of the reagents, placed in the instrument, and the galvanometer sensitivity is adjusted to yield a full deflection of 100 scale divisions. Because the cell acts as a condensing lens, its removal brings about a fall in the galvanometer deflection. This "air-setting" is noted and maintained throughout subsequent readings by adjustment of the galvanometer rheostat. The percentage light transmission of the unknown solutions can then be read directly as units of deflection on the scale.

Calculation. *For the Pulfrich Photometer.* If 0.2 ml. of serum is extracted in a 5 ml. flask, the concentrations of free and total cholesterol in milligrams per 100 ml. are calculated from the equations:

$$\text{Free cholesterol concentration} = \frac{1250 \times d}{L \times K_{mg.}} \qquad \text{Total cholesterol concentration} = \frac{2500 \times d}{L \times K_{mg.}}$$

in which L is the length of cell in centimeters, $K_{mg.}$ is the extinction coefficient per milligram of cholesterol, and d is the density (read directly in the newer instruments, or calculated from the percentage transmission (T) from the equation $d = 2 - \log T$). The value of $K_{mg.}$ should be determined independently by the operator with pure anhydrous cholesterol. Schoenheimer and Sperry found in their laboratory that $K_{mg.}$ was 1.450 (Table I).

For the photoelectric photometer. The calculation is the same as for the Zeiss-Pulfrich photometer provided that $K_{mg.}$ is constant over the range of measurement. If the filter pack does not isolate a sufficiently narrow segment of the spectrum, $K_{mg.}$ may vary somewhat and it will be necessary to prepare a calibration chart.

[In this laboratory we have used a Coleman Universal Spectrophotometer to read the color; reading per cent transmittance at a wave length of 620 mμ, using matched square curvettes of 13.06 mm. optical thickness. The total volume of solution was increased from 3 ml. to 6 ml. (the minimum volume for the Coleman curvettes of the above optical depth) by doubling the quantity of reagents used, as suggested by Schoenheimer and Sperry—2 ml. acetic acid, 4 ml. acetic anhydride, and 0.2 ml. H_2SO_4. The percentage transmittance as determined for amounts of cholesterol between 0.03 and 0.5 mg. is a straight line.]

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* When a great many determinations are to be made, it is worth while to prepare a chart on semilogarithmic paper by means of which amounts may be read off directly from photometer readings.

POLAROGRAPHIC METHODS

THE chemical methods commonly used in analysis of biological material are time-consuming and demand large amounts of sample. These factors preclude wide application of the procedure in connection with medical diagnosis and treatment. Accurate analytical methods which require small samples and may be quickly executed expand the possibilities of chemical analysis, both as an aid to the practice of medicine and as the tool of clinical research. In conjunction with studies of nutrition and chemical growth in childhood, we have applied polarographic and spectrographic methods in the determination of certain mineral elements in biological materials. Methods have been devised for determination of sodium, potassium, phosphorus,[334] and sulfur. In addition, methods have been evolved, thus far, for the determination of copper, zinc, iron, manganese, and nickel, and applied to metabolic samples.

In analysis of biological material the polarograph seems to possess some advantages over the spectrograph. The polarograph is a less expensive instrument and is especially useful in the simultaneous determination of several elements in a solution. Usually, separation of an interfering substance is easily obtained by using a regulating solution which causes the interfering substance to be precipitated or bound as a complex.

Emission spectrography is preferable to polarography when rapid qualitative analysis is required or when, in quantitative analysis, the spectrographic method can be applied directly to biological material. In such instances the procedures involved in preparing ash solutions of the material, which are essential to polarographic determinations, can be eliminated. For the quantitative determination of inorganic elements in feces, food composites, or special foods, the material usually must be treated in the same way for either method of determination.

The theory of polarographic microanalysis is well established,[185,186] however, there are many phases of the practical application which are still empirical and require trial adjustment. Mechanically, the polarograph is an easily handled instrument and the technique of operation may be mastered in a relatively short time, but performance of polarographic analyses, especially with biological material, requires considerable experience and results may be disappointing in the hands of an unskilled person. Perhaps for this reason, although the method has been known and used successfully for over fifteen years by a few workers, the polarograph has only recently found a prominent place in biological research. As with any physical method, however, application is limited to certain types of problems, and the proper estimation of the possibilities of the polarograph will eliminate unjust criticism of the method.

A negatively charged electrode, a cathode, acts as a reducing agent. If a gradually increasing negative potential is applied to a cathode immersed in a solution of several reducible ions, the most easily reducible cation will be deposited first and as the negative potential or reduction power is increased, the other cations present in the solution will be reduced as the cathode potential necessary to their reduction is reached. The reduction potential at which a reducible substance is deposited is, under definite conditions, a characteristic constant of that substance, by which its identity may be determined.

In electrolysis, electro-reduction takes place on the cathode and electro-oxidation on the anode, under the influence of the electromotive force (e.m.f.) applied to the electrodes. The current which flows through the solution is a measure of the reactions taking place on the electrodes. If a graph is obtained under proper conditions by plotting gradually increasing e.m.f. applied, against the corresponding current simultaneously transmitted (a current voltage curve), not only the reduction potential but also the concentration of a reducible substance in solution may be determined, provided influence upon the current, from the process taking place at the anode, is excluded.

During the reduction process the metal of the cathode can react with the element deposited and thereby change the chemical nature of the electrode, thus, constant reduction potentials over even a short period of electrolysis are not obtainable. In polarography, chemical changes of the cathode are excluded by the use of the dropping mercury cathode of Kucera,[190] which consists of a very narrow capillary from which small drops of mercury fall slowly into the soluton to be tested and provide continuous renovation of the mercury surface of the cathode.

Simultaneously with the reduction at the cathode, equivalent oxidation occurs at the anode. To exclude any influence of the anodic process, so that only the reaction at the cathode is measured, the anode of the electrolytic system consists of a relatively large, quiet pool of mercury. It was proved by Majer[226–228] that, in the presence of a dropping mercury cathode with small surface, the anode remains depolarized if its area is greater than 1 sq. cm. and if the solution contains ions (halide) which form insoluble salts with mercury. In polarographic analyses, only the dropping mercury cathode is polarized, for its surface is so small in comparison with that of the anode that the potential of anode remains practically constant and independent of the applied e.m.f.

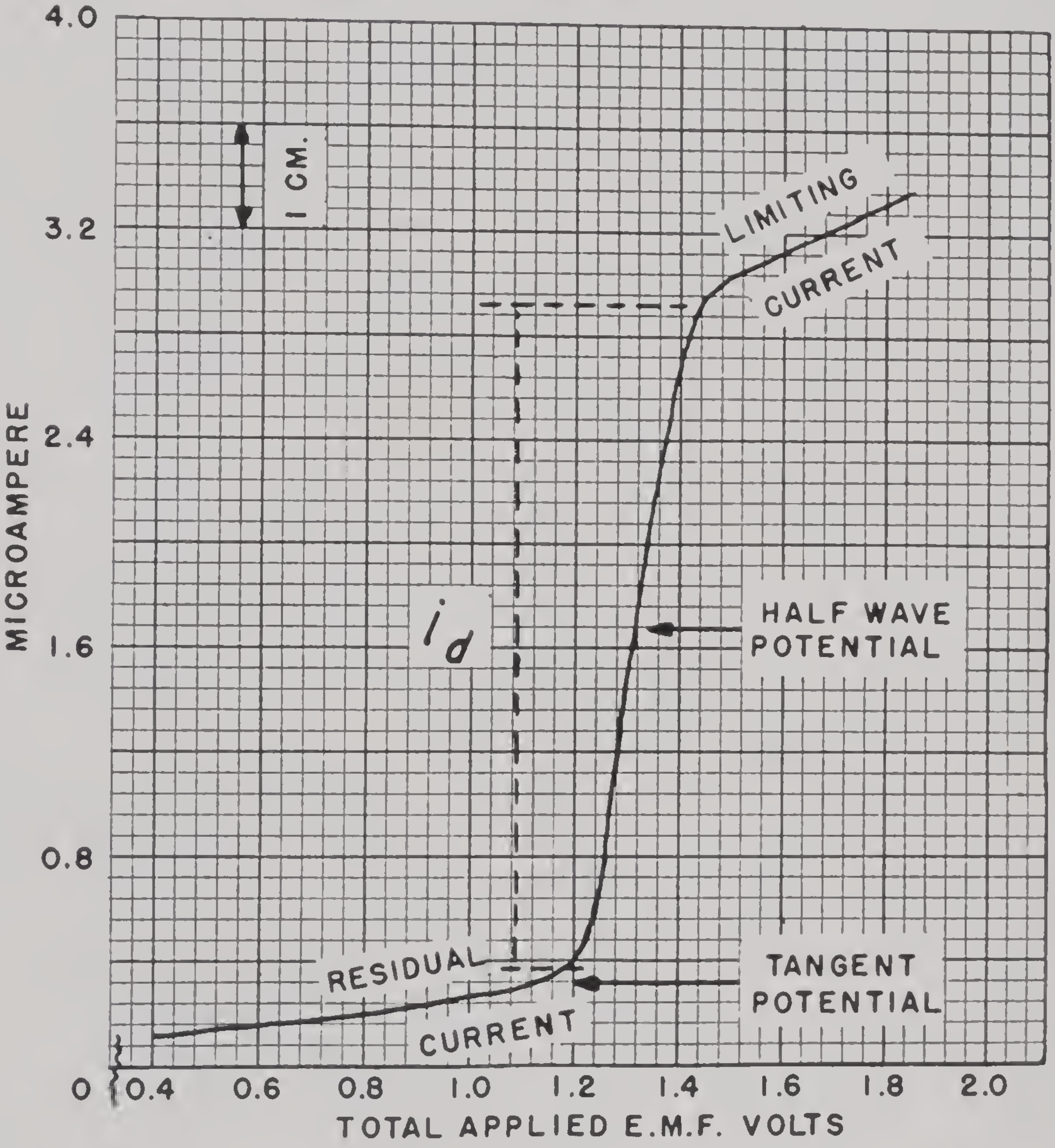

FIGURE 55. Current voltage curve of zinc in ammonium chloride solution.

Heyrovsky applied the dropping mercury cathode to obtain reproducible current voltage curves and his "polarographic method" of analysis is based upon the interpretation of current voltage curves so obtained. The current voltage curve (polarogram) shown in Figure 55 was determined with a solution of zinc sulfate containing ammonium chloride. Simultaneously the cathode potential is increased gradually from zero and the current transmitted through the solution is measured with a highly sensitive galvanometer. The recording is made so that the values of the potentials applied (volts) are plotted against the corresponding current transmitted (microamperes). The initial increase in current transmitted as the e.m.f. is increased (residual current) is very small, however, with the beginning of reduction of zinc the current increases rapidly. As the decomposition potential is exceeded, electrolysis continues and an extremely small amount of the ion (zinc) precipitates to form a dilute amalgam. The current does not increase indefinitely but, after a short time, reaches a limited value. This "limiting current" is constant and independent of further increases in potential. Under optimal and constant conditions, the increase in current (i_d) or the "wave height" of the "polarographic wave" is proportional to the concentration of the (zinc) ions, and its accurate measurement is the basis of quantitative polarographic analysis.

The limiting current can be explained by considering the reduction of dissolved reducible molecules in small concentration at a dropping mercury cathode of small diameter. When the potential reaches a value at which each molecule reacts as soon as it strikes the mercury surface of the cathode (reduction potential), the surrounding area of the mercury surface will be depleted with respect to the reducible molecules. This loss is compensated by diffusion of fresh molecules from the bulk of the solution. The rate of diffusion depends directly on the difference in concentration between the depleted area adjacent to the surface of the cathode and the bulk of the solution. The rate of diffusion, and hence the diffusion current, becomes constant when the concentration around the mercury surface is so small that the difference in concentration approaches a constant average value.

With neutral molecules in solution, the increase in current (i_d) is caused almost entirely by this diffusion. It is evident, then, that the magnitude of the diffusion current is a measure of the concentration of the reducible molecules. Keeping constant the factors which influence this diffusion current, it is directly proportional to the concentration of the reducible molecule in the bulk of the solution. In the reduction of ions, current not only is caused by diffusion but also by electrical migration. The migration current must be eliminated, since only the diffusion current is proportional to the concentration. This is achieved by the addition of an excess of indifferent salts,* so that the limiting current is determined entirely by diffusion. In the determination shown in Figure 55, ammonium chloride was used as the indifferent salt, or supporting electrolyte.

The amount of diffusion current at a given temperature, determined by the concentration of the reducible ion, is influenced by the diffusion coefficient of the ion, the mass of mercury discharged per second from the dropping mercury cathode, and the time in seconds per drop of mercury. Ilkovič[164] has derived an equation which embraces all these various factors:

$$i_d = 0.73 \times nF \times C \times D^{1/2} \times m^{2/3} \times t^{1/6} \text{ (microamperes)}$$

in which F is the Faraday constant in coulombs, C is the concentration of the ion (millimole per liter), D its diffusion coefficient (square centimeters per second), m the mass of mercury in milligrams discharged per second from the cathode, and t the time per drop of mercury. The equation clearly shows the extent to which these various factors influence the proportionality between diffusion current and concentration. It shows that, for a quantitative polarographic determination, the factors D, m, t, and the temperature, must be constant.

The diffusion constant, D, is dependent upon the frictional resistance of the solution, therefore, changes in the viscosity of the solution must be avoided, for quantitative comparison. Normally, the viscosity of a solution is sufficiently controlled when an approximately equal amount of supporting electrolyte is added to the solution in which the ions are to be determined. The flow of the mercury droplets must be constant and well-regulated, since this determines the constancy of the mass of mercury discharged per second. The same capillary must be used for comparative, quantitative analyses and its characteristics must be constant for calibration and determination. The diffusion current increases with increasing temperature about 2.5 per cent per degree; thus, temperature must be maintained within a range of one-half degree. With all these influencing factors constant, the diffusion current (i_d) is proportional to the concentration (C) of the reducible substance, and is measured in the current voltage curve as the height of the polarographic wave in millimeters of galvanometer deflection or in microamperes directly (Figure 55).

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* A salt which is reduced at a much more negative potential than the reducible substance to be determined and which does not influence in any way the ionic state of the reducible ion is called an "indifferent salt." Alkali salts and alkaline earths are reduced at a potential more negative than all the heavy metals and usually are used as indifferent salts for the reduction of these metals.

The identity of an electroreducible substance is indicated by its reduction potential, the point on the voltage axis where a rapid acceleration of current begins (Figure 55), however, the position of this point (tangent potential) varies somewhat with the concentration of the reducible substance. Heyrovsky and Ilkovič[136] have shown that the inflection point of a polarographic wave, given by the point on a current voltage curve where the current is equal to one-half of the value of its limiting current, is generally independent of the electrode characteristics and the concentration of the reducible substance. This "half-wave potential" is characteristic for the particular reducible substance in a definite solution if the value is referred to an external reference electrode of constant known potential (normal calomel electrode). The half-wave potential is used in qualitative polarographic determinations. Tables of half-wave potentials and tangent potentials for various elements have been published.[142,186]

In a solution of several reducible substances, each substance shows an individual polarographic wave if the reduction potentials are not too close together. For this reason, it is possible to determine quantitatively, as well as qualitatively, several elements in a single polarographic determination. The half-wave potential of an ion is not changed by the previous discharge of another ion and the wave heights generally do not influence each other, but have the same value as when determined alone in the same medium.

Heyrovsky and Shikata[134] constructed an instrument with which current voltage curves may be obtained automatically. Usually, the curves are recorded photographically. With the polarograph* a complete current voltage curve can be obtained in less than ten minutes.

At the beginning of a determination with the Heyrovsky polarograph (Figure 56), the moving contact, a small grooved wheel, is set at zero. The motor is started and as the moving contact traverses the bridge, gradually increasing voltage is applied to the dropping mercury cathode. As long as the voltage is less than the decomposition potential of the ion to be determined the flow of current is very small (residual current, Figure 55) and the light beam of the galvanometer remains practically stationary. Hence, the line that the light beam traces upon the paper is al-

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* The manufacturers of polarographs give excellent descriptions of the mechanism of their instruments. A Heyrovsky type polarograph is manufactured by E. H. Sargent and Company, Chicago, Ill. The Electro-Chemograph is constructed by Leeds and Northrup Company, Philadelphia, Pa. and Fisher's Electropode (for manual determination), by Fisher Scientific Co.

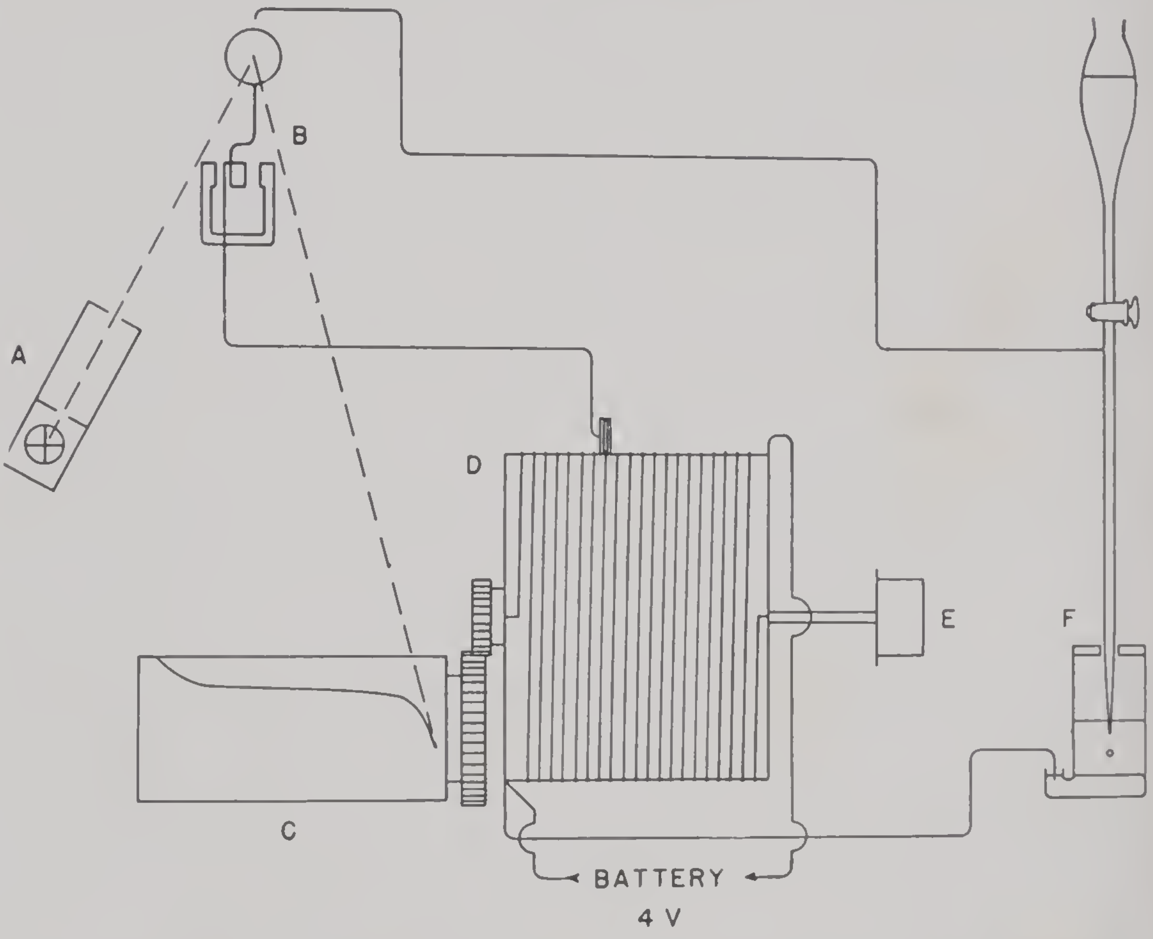

Figure 56. Schematic Representation of the Heyrovsky Polarograph. The dropping mercury cathode (F) is connected with the electrolysis cell containing the solution to be analyzed. The height of the reservoir of mercury to which the dropping mercury cathode is attached can be adjusted, thereby changing the speed at which droplets are released from the capillary. The potentiometer bridge (D) is a cylinder wound with exactly 20 turns of low-resistance wire. Current is supplied to the bridge by a storage battery of 4 or 2 volts; thus, the increase in voltage from one revolution of the bridge is 200 or 100 millivolts, respectively. A cylinder (C) carries the photographic paper on which the current voltage curve is recorded and is connected with the potentiometer bridge by a system of gears. Both cylinders are rotated simultaneously by a slow, uniform-speed motor (E), so that one revolution of the potentiometer bridge (200 to 100 *mv.*) exactly corresponds with 1 cm. on the recording paper. A beam from the light source (A) is reflected upon the recording paper by the mirror of a galvanometer (B). The sensitivity of the galvanometer usually used is about 2.0×10^{-9} amp./mm./m., which can be changed by Ayrton shunts connected into the circuit in parallel.

most parallel to the circumference of the registering cylinder. When the reduction potential is reached, the current transmitted increases immediately, the light beam from the galvanometer moves rapidly across the paper (Figure 55, i_d) until the limiting current is reached, then resumes tracing a circumferential line.

A device for manual determination of current voltage curves by determining a series of points which indicate the curve has been described by J. J. Lingane and I. M. Kolthoff.[203] This method is precise but slower and, for this reason, is not suitable for routine analysis. Furthermore, by automatic registration, a continuous curve is obtained and small curves can be seen which might not be detected with the manual device.

Dropping mercury cathode: The dropping mercury cathode consists of a thermometer capillary tubing of Pyrex glass, 8 to 10 cm. long, approximately 5 mm. in outside diameter and 0.4 mm. in internal diameter, drawn out at one end into a tip at least 2 cm. long with an internal diameter of about 0.03 mm. The small end of the capillary should have an outside diameter of 2 mm. The exact dimensions which will provide a suitable drop rate for the range of potentials in a given electrolyte solution must be determined by trial. Maas[207] and Siebert and Langer[315] have described the construction of suitable capillaries. The capillary is connected with a mercury reservoir with neoprene rubber tubing, which if carefully cleaned does not contaminate the mercury.

By changing the height of the mercury reservoir over the tip of the capillary the rate at which droplets fall from the tip of the capillary may be altered. Normally, a mercury droplet should fall into the solution to be analyzed every three seconds. The drop rate is determined not only by the height of the mercury reservoir, but also by the bore and length of the capillary, the purity of the mercury and the interfacial tension between mercury and the electrolyte. The capillary characteristics must be constant for quantitative comparison, *i.e.*, the same capillary must be used and the height of the mercury reservoir, once adjusted, must be kept constant. Only under these conditions is a constant mass of mercury discharged per second from the cathode, a requirement for linear proportionality between diffusion current and concentration (p. 356). For comparative polarograms the tip of the capillary should be immersed to the same depth (at least 3 mm. below the surface) in the electrolyte solution and should be located similarly with respect to the anode surface of the electrolytic cell, especially when working with micro-electrolysis cells. The capillary must be handled carefully and dipped into the solution only after the connection between mercury reservoir and capillary has been opened for some time, in order to avoid entrance of the liquid into the bore of the capillary. After use, the outside of the capillary should be dried with filter paper and some mercury allowed to escape before the tip is immersed in pure mercury and the connection with the reservoir closed. The bore of the capillary should never be allowed to become wet.

During the formation of a mercury droplet at the cathode the current changes between a minimum and a maximum value, therefore, the galvanometer used to measure the current must have a relatively long period (15 sec.) so that only an average value is recorded and only very small oscillations are produced in the current voltage curve. Large oscillations make evaluation of the current voltage curve difficult and reduce the accuracy of the determinations.*

Normally, a slow dropping cathode shows greater oscillation than a faster one. The drop time, however, must not be so fast that the droplets are unequal in size or stir the liquid, preventing a steady transmission of diffusion current. The drop time changes somewhat with increasing potentials, a fact which must be considered in determinations with alkali metals. Such elements should be determined with capillaries possessing a very slow initial drop time but which will attain, at the reduction potential (−1.8 to −2.2 volt), a rate of two to three seconds per drop.

The amount of mercury used for one determination is very small, hence, the level of the mercury reservoir and the distance to the tip of the capillary remain practically unchanged. After many determinations, absolutely pure mercury must be added to maintain the level in the reservoir.† It is advisable to check the purity after double distillation, by obtaining a current voltage curve of an indifferent salt solution which does not contain the ion to be determined, using the sensitivity to be employed in actual determinations.

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* Lingane and Kerlinger[204] have described a condenser which reduces galvanometer oscillations and is frequently helpful.

† Mueller[213] has described a dropping electrode with a constant head of mercury.

Polarographic cells: In most determinations which can be performed in air, a small beaker, or weighing bottle, may be used as a cell. The anode consists of a layer of mercury about 4 mm. deep on the bottom of the cell. The electrical connection to the anode is made with a platinum wire sealed within a glass tube so that only a small loop of the wire at the end is not covered. The platinum contact is immersed in the mercury so that the wire does not touch the bottom of the vessel but is completely surrounded with mercury. For determination of comparative polarograms, vessels of the same size should be used if the concentrations are very low. If the determination must be carried out in the absence of oxygen, special cells are available which have an inlet through which hydrogen or nitrogen can be bubbled through the solution to remove dissolved oxygen. A cell which has proved satisfactory in our work is shown in Figure 57. Other types of electrolytic cells are pictured in the monograph of Hohn,[142] and Majer[225] has described polarographic cells particularly suited for microanalytical work.

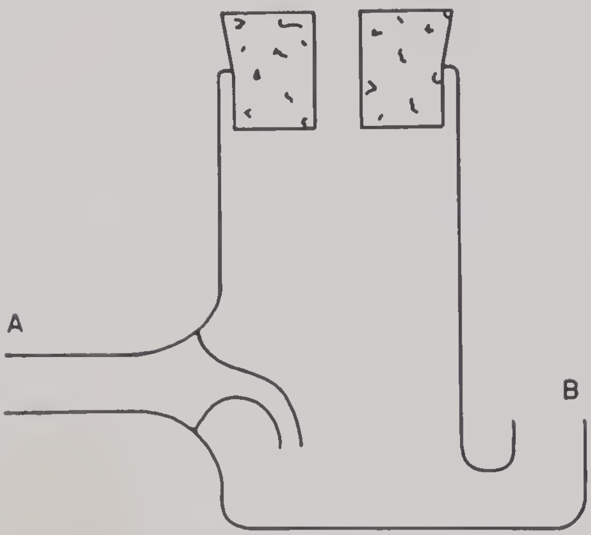

FIGURE 57. Electrolysis cell. Hydrogen or nitrogen is introduced by the capillary side tube A. The electrical connection to the quiet pool of mercury (anode) is made by immersing the platinum contact in the side arm of the cell, B, which is partly filled with mercury. The mouth of the cell is closed with a cork stopper, containing a hole into which the capillary fits perfectly. This hole serves also as an outlet for the hydrogen. After hydrogen has bubbled through the solution for sufficient time to remove the oxygen, the capillary is inserted in the hole quickly and the stream of hydrogen stopped. Penetration of oxygen into the cell after the hydrogen is shut off is practically excluded. The flow of hydrogen bubbled through the solution should not be too strong; however, it should be fast enough to remove the oxygen in approximately 20 minutes.

Temperature: Since the diffusion current increases with the temperature, the latter must be controlled within one-half degree. In certain instances the use of a thermostat to achieve the necessary constancy of temperature cannot be avoided, however, its use is not convenient for routine analysis. In almost every laboratory a room may be found which is so situated that the temperature does not change rapidly and is rather constant over lengthy periods of time. Each time a change in temperature occurs a new calibration must be made. When enough calibrations have been made to cover the range of the normally occurring changes in room temperature, corrections for temperature changes may be made without making a new calibration. By this procedure error attributable to temperature changes is practically excluded.

Regulating solution: In a solution which contains only a reducible salt, the current is carried entirely by the ions of the salt. The limiting current obtained represents the total current produced by both diffusion and migration. If a small amount of an indifferent salt is added to the solution containing the reducible salt, the limiting current decreases rapidly. The limiting current becomes practically constant and independent of further addition when sufficient indifferent salt has been added so that the current through the solution is carried entirely by the ions of the indifferent salt, eliminating electrical migration of the reducible ions (p. 341). For this reason the solution in which a reducible substance is determined must contain a large excess of an indifferent salt. Furthermore, it must be so composed that the substance to be determined is held in definite ionic or molecular form such as a hydrated ion or a definite complex ion, not as a colloid or in equilibrium with other complex ions present. The wave must have sufficient space and not be obscured by other reduction or precipitation reactions occurring during the electrolysis. The solution must be so composed, by addition of a colloid or changing of concentration of indifferent salts, that the wave does not show a maximum caused by adsorption forces.

An electrolyte solution which regulates all these requirements of accurate and unobjectional performance of a current voltage curve is called a regulating solution. The principal difficulty in working out a polarographic method of determining an element in biological material lies in finding a suitable regulating solution, one in which the element can be determined without any interference from other substances which may be present and which provides quantitatively comparable waves.

Current voltage curves for quantitative determinations must be obtained under identical conditions to keep constant all the various influences upon the wave height of the curve. The concentration of the indifferent salt must be approximately equal, so that the activity coefficient of the ions to be determined remains practically constant in solutions of different concentrations. This is accomplished by adding a small amount of the test sample, volumetrically, to relatively large volumes of regulating solution, containing at least 10 times greater concentrations of indifferent salts than the concentration of the ions to be determined. Calibrations are performed in the same regulating solution and in the same manner, thus eliminating any influence of the concentration of the indifferent salt.

The choice of the indifferent salt to be used depends on the properties of the ions to be determined and other elements which are present. Usually, alkali salts are employed since they have a high negative reduction potential. For the determination of the alkali metals themselves, tetramethyl-ammonium-hydroxyde in ammoniacal solution is used. A principal advantage of the polarographic method lies in the fact that the regulating solutions can be so composed that other elements, the presence of which is undesirable, will be precipitated. In neutral solutions the reduction potentials of iron and manganese are so close together that the two waves coalesce, giving approximately the sum of the iron and manganese waves in one single wave. In ammoniacal solution, in the presence of ammonium chloride, iron is precipitated and only the wave of manganese appears. It is not necessary to filter off the iron hydroxyde, and the manganese can be determined in the presence of the iron without any previous separation.

A simultaneous determination of two ions, the reduction potentials of which are so close together that their waves coalesce, can be achieved by using a regulating solution which contains a salt that forms complex compounds with these ions and thus separates the reduction potentials. Two distinct waves for cobalt and nickel may be obtained in a solution containing potassium thiocyanate, whereas the two waves coalesce in a solution of ammonium sulfate. It is evident that a great variety of regulating solutions can be composed for various purposes by using different inorganic salts as indifferent salt, by using a special *pH*, or by using organic solvents. Hohn[142] has given a list of regulating solutions for various determinations and some applications are reported by Thanheiser and Maasen.[343]

Maxima: Current voltage curves do not always present the ideal shape (Figure 55), which is essential for accurate determination of the wave heights. Very often the curves show a sharp rise and a sudden decrease at the point where the limiting current gradually develops, that is, they show a "maximum." The occurrence of a maximum depends on various factors and is caused, according to Heyrovsky,[135] by adsorption of the electroreducible substance on the growing mercury drops, increasing the concentration of the reducible substance above the value in the bulk of the solution and thus preventing a normal concentration polarization. It is absolutely necessary to eliminate these maxima in quantitative analysis, for there is no linear relationship between height of maximum and concentration of the reducible substance.

Maxima usually can be eliminated by adding traces of capillary active electrolytes and non-electrolytes, various capillary-inactive ions and charged colloids. Substances commonly used for this purpose are methyl cellulose, gelatin, starch, fatty acids, and dyestuffs (methyl red). The selection of a maximum suppressor depends on the reducible substance to be determined and on the indifferent salt used. Neither should react with the maximum suppressor, which should not be electroreducible or give a polarographic wave at the dropping mercury cathode. Methyl red is electroreducible, as are most dyestuffs, however, the amount applied is extremely small and will not interfere with the wave of the reducible substance to be determined. The amount of suppressor added should be as small as possible; in most cases addition of a few milliliters of a solution containing 2 gm. of gelatin or methyl cellulose per liter will suffice to suppress a maximum, but the quality and quantity necessary under particular conditions must be found by experiment.

Sometimes maxima can be suppressed by increasing the concentration of the indifferent salts employed. Adding traces of a colloid often helps to stabilize the drop rate of the dropping mercury electrode. Oscillations of the current, which may occur to a higher degree in some regulating solutions, can be greatly reduced by adding a colloid. Since the diffusion current depends upon the diffusion coefficient of the reducible ion, the drop rate and the mass of mercury which leaves the cathode per second, it is necessary that the amount of colloids or capillary-active ions added to the regulating solution as a maximum suppressor is exactly the same for comparative analysis. In a series of comparative analyses, after preparing a new batch of regulating solution which contains a colloid, it is always advisable to perform a new calibration with the new regulating solution.

Simultaneous determination of several ions: Determination of several electroreducible substances in one operation requires a regulating solution in which all components to be determined exist in a definite form. The reduction potentials should not be too close together, for sufficient displacement between successive steps must be available in order to achieve a complete wave for each component without interference from the wave of the next reducible substance. The space required for the wave of a discharging ion varies with the nature of the ions in solution and with the concentration of the reducible substance. The concentrations of the elements to be determined should be similar and the substance with the highest concentration should be the one with the most negative reduction potential.

If the potential of a component with low concentration is more negative than that of one with a higher concentration, the first component can be determined with a sufficiently high galvanometer sensitivity and immediately following, the second component can be determined with reduced galvanometer sensitivity on the same record. If, however, the concentration of the component which reduces first is extremely high and the concentration of the next reducible substance is very low, the latter cannot be determined with accuracy, if at all. With the galvanometer sensitivity necessary for accurate determination of the component of small concentration, the wave of the constituent with higher concentration transmits such a large amount of current that the wave cannot be recorded on the polarogram. In such determinations the regulating solution for the determination of the second component must be changed so that the component with the high concentration is eliminated. Sometimes it must be removed chemically, although the separation does not need to be quantitative. It is sufficient to reduce the concentration of the element in question only to an amount which makes simultaneous determination with the other component possible.

An undesirable high wave can also be removed, according to

Ilkovič and Semerano,[163,300] by compensating for the diffusion current of the interfering major component with a current of the same magnitude, sent through the galvanometer in an opposite direction. By this procedure, the high wave is suppressed and the low wave of the other component can be determined with sufficient accuracy. Oscillations however, sometimes make the compensation method difficult.

Residual current: Before using a regulating solution it must be proved to be free of the substance to be determined. A current voltage curve is made with the regulating solution alone, under the same conditions and with the same galvanometer sensitivity to be used for the analytical determination. The curve for the regulating solution shows a small, uniform increase attributable to "condenser current" and often to some "faradic current" from impurities, which continues until the beginning of the sharp rise in current produced by discharge of the ions of the indifferent salt. This small "residual current" must be subtracted from the observed total current of a reducible substance in the same regulating solution, for the correct quantitative evaluation of the diffusion current.

Knowledge of the residual current is most important in the determination of substances in very small concentrations, wherein a high galvanometer sensitivity must be employed. In such solutions, the residual current is high in comparison to the diffusion current of the reducible substance; sometimes, it reaches values which make the detection of very small amounts of substances impossible. For "trace analysis" with the polarograph, the residual current can be reduced or completely eliminated by compensation with a current of the same magnitude sent through the galvanometer in opposite direction,[163] as described for suppressing high waves which may interfere.

Foreign waves: The regulating solution should not give any foreign wave belonging to an element which occurs as a constituent of the regulating solution itself or in the region where the wave of the substance to be tested appears. Using a very high galvanometer sensitivity for the examination of the regulating solution, two waves are generally observed which are caused by the reduction of the oxygen of the air dissolved in the regulating solution. Since these waves usually occupy a large space in the polarogram, they are very disturbing and must be removed for determination of most metals in low concentrations. The air can be removed by bubbling an inert gas through the regulating solution (hydrogen or nitrogen), in a special electrolytic cell (Figure 57). Traces of oxygen must be removed from the inert gas by washing with an alkaline solution of pyrogallic acid (36 gm. NaOH and 3 gm. pyrogallic acid in 100 ml. of water).

Removal of the air requires 15 to 30 minutes, depending upon the concentration of oxygen in the solution. The gas stream must be stopped during the electrolysis; its stirring effect would cause fluctuation in the current. Regulating solutions which are almost saturated with indifferent salt contain little air, and, with them, the removal of oxygen can be avoided in many instances. Oxygen removal is unnecessary in determinations involving higher concentrations of reducible substances, for only low sensitivity of the galvanometer is required, and waves of the small amount of oxygen are not recorded. The oxygen removal procedure with inert gas is time-consuming and chemical means have been proposed for the removal of the oxygen. Addition of a very small amount of sodium sulfite (or manganese salt in alkaline solutions) removes the oxygen rapidly, however, this method can be used only if the sulfite (or manganese hydroxide) does not react with one of the components of the regulating solution or with the reducible substance itself. Generally, chemical removal of oxygen is not recommended.

In acid regulating solutions, hydrogen also may cause undesirable waves, usually appearing as high maxima, since the high over-voltage of hydrogen at the cathode can be decreased in the presence of a small amount of metals. Especially with iron compounds, the *pH* of the acid regulating solutions, used in the determination, should not be below 4, otherwise a proper proportionality between wave height and concentration is difficult to obtain. With reduction potentials below 1.0 volt a catalytic discharge of hydrogen in acid solution usually does not occur. If possible, however, metals of the iron group should be determined in neutral or alkaline solutions using, if necessary, complex-forming agents (oxalic acid, tartaric acid), to keep the metals in solution.

Calibration and measuring of wave heights: Since the diffusion current of a reducible substance is proportional to its concentration in a solution, all samples must be defined volumetrically for quantitative analysis. To determine the concentration of a reducible substance in a series of samples, a calibration curve has to be performed with known amounts of the substance in question. Since the calibration is performed so that, each time, the various amounts of the element are dissolved in the same volume, the wave heights are proportional not only to the concentration but also to the actual amounts in the solution. By plotting the wave heights in millimeters against the correspond-

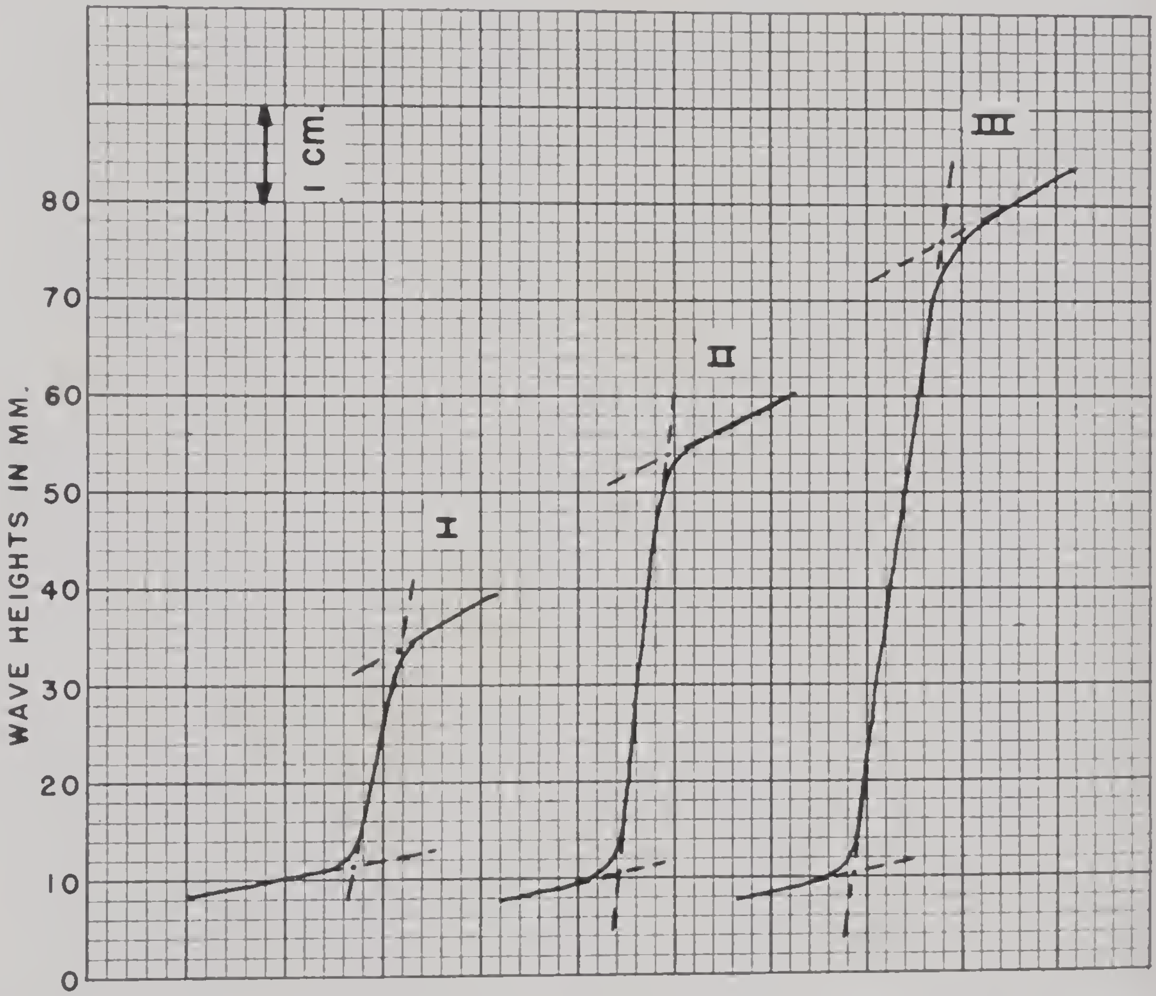

FIGURE 58. Polarograms for the calibration curve for zinc determination. Polarographic curves of standard solutions containing 0.135, 0.270 and 0.405 mg. of zinc per milliliter. The polarograms were taken with a galvanometer sensitivity of 1/30. Temperature, 26° C. The diffusion current is sometimes given directly in microamperes, which is preferable for theoretical considerations. For practical quantitative measurement, measuring in millimeters of galvanometer inflection is most convenient.

ing amounts of zinc, a calibration curve is obtained, by means of which unknown amounts can be readily determined. The polarograms for a calibration curve for zinc are shown in Figure 58. The calibration curve obtained in most cases is a straight line which intersects the abscissa close to the zero point (Figure 59).

The determinations of unknown amounts of a substance in biological material must be performed in exactly the same manner as the calibration. It is carried out by transferring 1 ml. of the ash solution of the biological material into a 10 ml. volumetric flask and filling up to the mark with the regulating solution. The amount of zinc in 1 ml. of the ash solution should be within the range for which the calibration was performed. The increase in neutral salts in the regulating solution by addition of 1 ml. of the ash solution is negligible. The polarogram is taken under the same conditions (the same capillary, temperature, etc.) as the polarograms for the calibration; the wave height is measured, and the amount of zinc determined from the calibration curve.

In instances in which more than 1 ml. of an ash solution must be used to insure a sufficient amount of the substance to be determined, a volumetrically defined amount of ash solution is carefully dried and dissolved directly in the regulating solution. The increase in neutral salts must be controlled and, if too large, must be considered by the calibration, which must be carried out under the same conditions as the determination with the unknown.

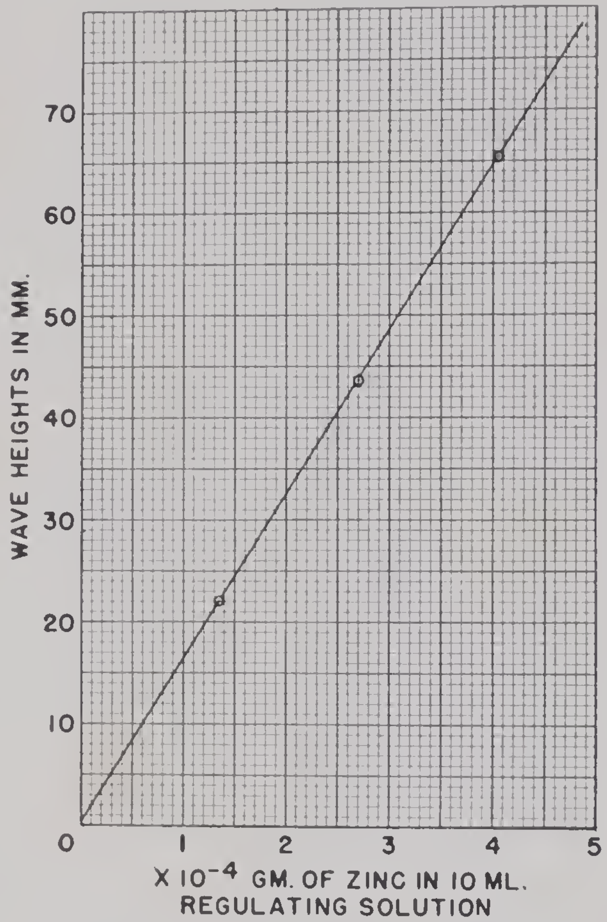

FIGURE 59. Calibration curve for zinc determination. Galvanometer sensitivity 1/20, Temperature 26° C.

If only one determination for an element is to be made, a calibration curve can be avoided by using an internal standard. The polarogram of the unknown solution is obtained with a suitable regulating solution. Another polarogram is made after adding to the unknown, 1 ml. of a standard solution containing a known amount of the element. From the wave height given by the unknown solution and the increase in wave height caused by the addition of the known amount of the reducible element, the concentration of the element in the original unknown solution can easily be calculated.* This type of calibration has an advantage, inasmuch as the added amount is determined in the presence of the salts in the original solution, however, the volume of the original solution is increased by addition of the standard solution.† To avoid the change in volume we have employed the following procedure. One milliliter of an ash solution is dried, dissolved in exactly 10 ml. of the regulating solution, and the polarogram taken. Another 1 ml. sample of the ash solution, plus an added amount of a known standard solution, is dried and the residue dissolved in 10 ml. of the regulating solution and a polarogram taken. From the waves of these two polarograms the unknown amount in the original ash solution can be determined by simple proportion. This method is accurate and can be applied if enough ash solution is available.

The measurement of the "wave heights" is important for quantitative analysis. The definition of the wave height is not simple, since a polarographic curve does not often show very sharp curves at the beginning and the end of the wave, and the final diffusion current may be partially superimposed upon the beginning of the next wave. The wave heights can be measured by (1) measurement of the distance between points of contact of two tangents drawn at a 45° angle,[137] or a 35° 16′ angle,[300] to the curvatures of the wave; (2) measurement of the distance between two straight lines drawn perpendicular to the abscissa, one at the beginning of the lower curvature and one at the end of the upper curvature; (3) measurement of the distance between the

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* Formula for the calculation:[142]

$$x = \frac{A \times m \times \text{ml.}_{+} \times \text{ml.}_{1} \times \text{ml.}_{2} \times W_1}{1000 \times \text{ml.}_{2}(W_2\text{ml.}_{2} - W_1\text{ml.}_{1})}$$

In which: x = amount to be determined in the ash solution in grams.
A = atomic or molecular weight of the substance in question.
m = molarity of the added standard solution.
ml._{1} = volume of the original ash solution used.
ml._{+} = added volume of the standard solution.
ml._{2} = volume of the original ash solution used plus volume of added standard solution.
W_1 = wave height of the original ash solution.
W_2 = wave height of the original ash solution plus added standard solution.

† This method sometimes gives wrong values if the regulating solution contains a colloid, the concentration of which is lowered by addition of the standard solution, since the concentration of the colloid exerts a strong influence upon the wave heights.

points of intersection of three straight lines drawn coincident with the residual current line, the limiting current line, and the diffusion current line (the wave itself). The second method is reliable only with very sharp curves. The third method, known as the intersection point method, is shown in Figure 55 (dotted lines). It is generally applicable and is widely used in practical polarography. Which method of measuring the wave heights is employed does not usually matter, however, it is essential that the same method be used for comparative analysis.

Application of polarographic methods to biological material: To the nutritionist and physician the foregoing technique may seem very difficult and beset with many opportunities for error. The polarograph and spectrograph are both instruments of precision which can be used satisfactorily by one who has been specifically trained to do this type of analysis; but in dealing with biological materials the operator must be able to recognize interferences which frequently arise. To such an individual the outline of technique given herein will appear simple and be of assistance in carrying out analyses, but for a full discussion of the theory and details of the methods, original works and comprehensive treatises should be consulted. We have given more space to the discussion of polarography than to other, equally important procedures, because of recent adaptation to metabolic and biological materials and their broad application to problems of health and disease. Our own investigations with the polarograph and spectrograph, applied to metabolic studies, are still incomplete but some of the methods which have proved feasible in metabolic balance studies of the heavy metals are briefly represented on the following pages. These illustrate the application of polarographic methods and may serve as a stimulus to further exploration of the use of these instruments in biological studies.

The polarograph provides a valuable means of approach to a great variety of problems in biochemistry. It is suitable for the determination not only of inorganic elements but also of organic substances such as cystine,[35,36,285,332] homocystine[333] and a great variety of electroreducible substances such as vitamins,[57,183,205,392] and ketonic steroids.[74] Limitation of space prohibits the theoretical discussions involved in complete presentations of polarographic methods. These require separate publication of the detailed development of the methods and the results obtained in recovery experiments. An indirect method for the determination of phosphorus in biological material has been published.[334] Papers upon methods for the determination of sulfur in food, urine, and feces, and for the joint determination of sodium and potassium are in preparation. Polarographic or spectrographic methods of analysis for calcium, magnesium and other elements are being developed. These instruments enable us to reduce the time required for analyses, thereby offering the first possibility for development of practical, clinical application of metabolic balance study, although some may require expenditure of considerably more time in preparing to use the methods with biological material.

Preparation of food, urine and feces for polarographic analysis: Mixed food composites and feces were oven-dried at 60° C. Twenty and 5 gm. samples, respectively, of the dried material were ashed in platinum crucibles at 400° for approximately 10 hours. The ash was dissolved in concentrated hydrochloric acid, filtered into a 100 ml. volumetric flask, the residue re-ashed, dissolved in 5 ml. of concentrated hydrochloric acid, filtered into the same flask and the volume made up to 100 ml. with distilled water. Under vacuum of 150 to 200 millimicrons, 250 to 300 ml. of urine were dried from the frozen state and the dry material ashed, dissolved in hydrochloric acid, filtered into a 50 ml. volumetric flask and the volume made to the mark.

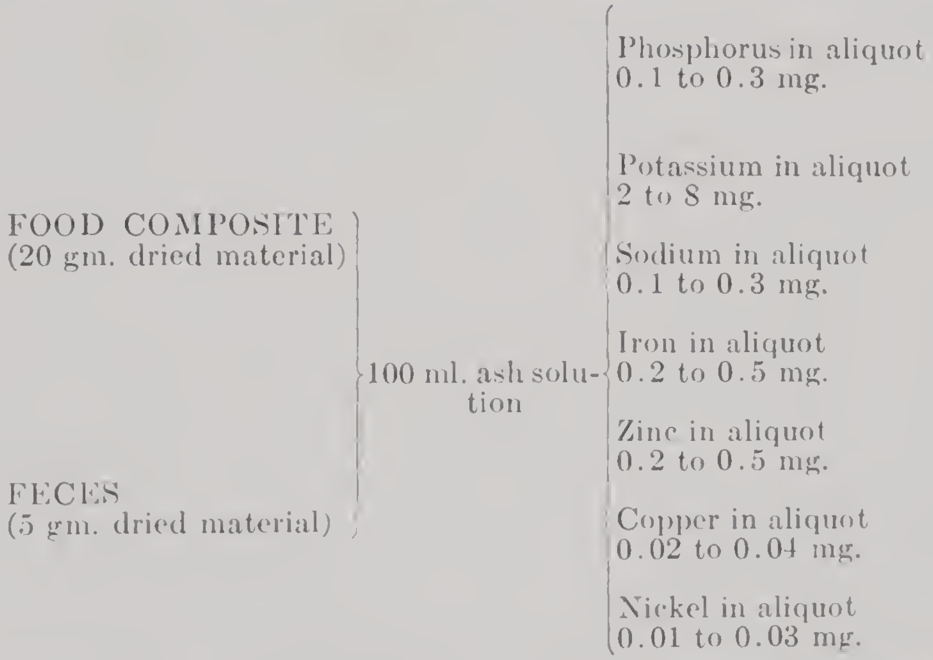

FIGURE 60. Required range of concentration necessary in aliquots of ash solution for polarographic analyses.

Figure 60 shows the small amounts of ash solution necessary for accurate analyses of feces for phosphorus, sodium, potassium, iron, copper, zinc, and nickel. It is difficult, however, to mix feces or food composite so well that a homogeneous sample of such minute quantity can be taken. For precise results, larger amounts should be ashed and small portions of the ash solution taken for analysis. After the ash solution has been prepared and the necessary calibrations made, actual analysis for the various elements is rapid. Simultaneous determination of copper and zinc on the ash solution requires about 10 minutes. The same amount of time suffices for the estimation of nickel. In determining phosphorus, sodium, potassium and iron, microchemical separations are involved. Sodium and potassium analyses can be carried out in about 2 hours; iron can be quantitated in one and one-half hours; the precipitation of phosphorus requires standing over night.

Analysis of Food Composites and Feces

Nickel

Calibration curves: Place 1 or 2 ml. of various standard nickel solutions in 10 ml. volumetric flasks. Add exactly 2 ml. of the gelatin regulating solution,* dilute to volume with the thiocyanate regulating solution† and mix thoroughly. (Small variations in the amounts of thiocyanate solution used (6 to 8 ml.) make no difference in the wave heights.) Place approximately 5 ml. of the solution in the polarographic cell and, after bubbling hydrogen through the liquid for 20 minutes, perform the polarogram. Polarograms of different standard nickel solutions in the regulating solution given above and the corresponding calibration curve are shown in Figure 61.

Feces: Dry 20 ml. of ash solution, carefully, in a platinum crucible. Add 8 ml. of potassium thiocyanate regulating solution† and mix thoroughly with a rubber-tipped glass rod. Macerate all lumps to assure solution of all the nickel present in the sample, then add exactly 2 ml. of gelatin regulating solution;* stir thoroughly and, if necessary, filter through a small filter paper. Place approximately 5 ml. of the solution in a polarograph cell; remove oxygen by passing hydrogen through the solution for about 20 minutes; then take the polarogram.

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* Gelatin regulating solution: Dissolve 0.1 gm. pure gelatin (Eastman Kodak) in about 100 ml. of water, warming gently. Filter into a 200 ml. volumetric flask and fill to the mark with water.

† Potassium thiocyanate regulating solution: Dissolve 3 gm. potassium thiocyanate and 60 ml. concentrated ammonia in distilled water in a 500 ml. volumetric flask and fill to the mark.

Food: Dry carefully 20 ml. of the ash solution of food composite in a platinum crucible and dissolve the residue in 5 ml. of regulating solution.* Then place the solution in the electrolytic cell and, after removal of oxygen with hydrogen, record the polarogram with a galvanometer sensitivity of one-fifth.

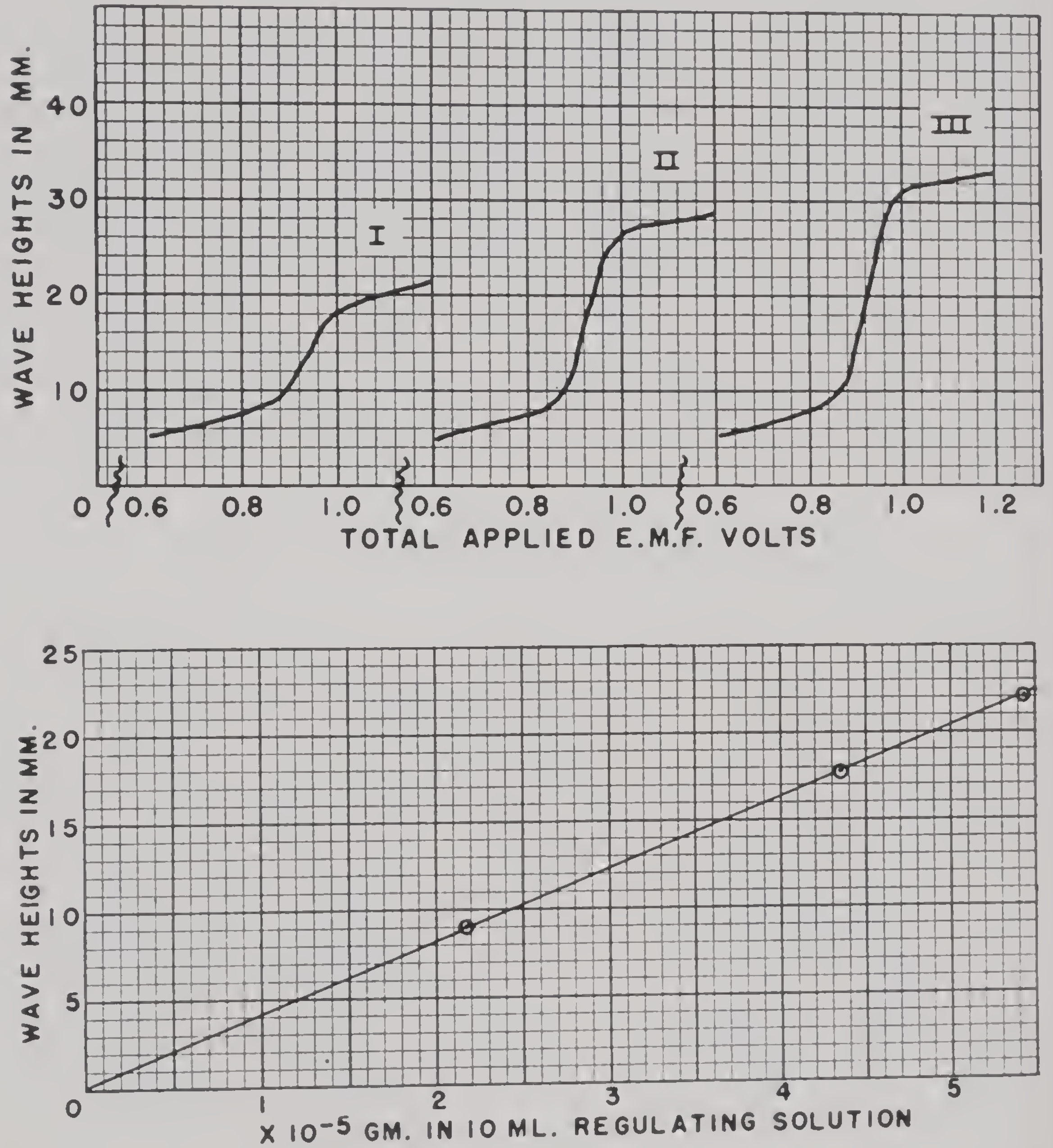

FIGURE 61. Polarograms of standard nickel solutions in ammonium thiocyanate regulating solution and the resulting calibration curve. Galvanometer sensitivity 1/10. Temperature 26° C.

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* Potassium thiocyanate regulating solution: 0.1 N ammonium acetate and 0.025 N potassium thiocyanate.

Copper and Zinc

Calibration curves: Place 1 ml. of each of three standard copper solutions* containing 0.0614 mg., 0.0307 mg., and 0.0150 mg. of copper, respectively, in a 10 ml. volumetric flask and add 1 ml. of each of three standard zinc solutions* containing 0.135 mg., 0.270 mg., and 0.405 mg. of zinc, respectively, to each 10 ml. volumetric flask. Fill the flasks to the mark with ammoniacal regulating solution† and determine the polarograms of these solutions after the removal of oxygen with hydrogen. Record the copper waves using a galvanometer sensitivity of 1/5 or 1/10; then, return the light beam of the galvanometer to the zero line

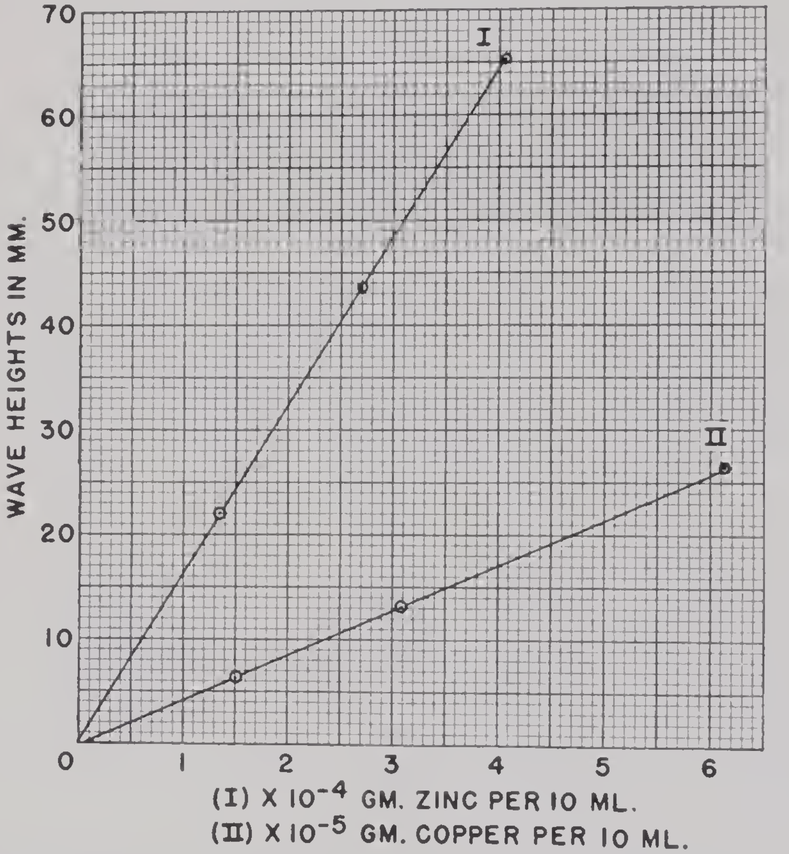

FIGURE 62. Calibration curves for simultaneous determination of copper and zinc. Galvanometer sensitivity 1/20. Temperature 26°C.

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[*] Prepare standard solutions of copper by dissolving $CuSO_4 \cdot 5H_2O$ (C. P. Baker's Analyzed) in distilled water and determine the copper content electrolytically. Zinc standard solutions were prepared by dissolving known amounts of zinc metal of highest purity in hydrochloric acid.

[†] Regulating solution: In a 1000 ml. volumetric flask, dissolve 50 gm. ammonium chloride in about 200 ml. of water and add 100 ml. concentrated ammonia. Dissolve 12 gm. of dextrin (pure "Dextrin Merck") by heating in about 100 ml. distilled water. When cool, filter the dextrin solution into the solution of ammonium chloride and ammonia and fill to the mark. Allow regulating solution to stand 15 hours or more before using.

of the recording paper and record the zinc waves using a galvanometer sensitivity of 1/20. Plot the wave heights against the concentration to obtain calibration curves for copper and zinc (Figure 62).

Feces: Place 10 ml. of the ash solution of feces in a platinum crucible and very carefully evaporate to dryness. Dissolve the residue in exactly 10 ml. of the ammoniacal regulating solution. Filter quickly, transfer 5 ml. of the filtrate into the electrolytic cell and determine the polarograms after removal of oxygen with hydrogen.

Register the copper wave starting with zero applied e.m.f. with a galvanometer sensitivity of one-fifth. After a sufficient part of the limiting current is recorded, move the light beam of the galvanometer back to the zero line on the recording paper and record the zinc wave with a galvanometer sensitivity of 1/20.

Food: Evaporate 15 ml. of ash solution in a platinum crucible and dissolve residue in 10 ml. of the ammoniacal regulating solution. Filter and use 5 ml. of this solution to determine the polarograms as described for feces.

Iron

To a suitable aliquot of the ash solution of feces or food composite (containing 0.1 to 0.5 mg. of iron) add concentrated ammonium hydroxide until the solution is slightly ammoniacal. After standing, the iron is completely precipitated. Co-precipitation of copper or nickel is minimized by the presence of ammonium chloride, formed by the HCl and NH_3 present in the solution. Neutral salts such as Ca_3PO_4, which sometimes precipitate in ammoniacal medium, do not interfere. Filter the solution and carefully wash the precipitate with ammoniacal water, several times. Dissolve the residue in a few milliliters of hot 2 *N* HNO_3 and filter the solution into a 10 ml. volumetric flask. Fill the flask to the mark with 2 *N* HNO_3, mix, then place 1 ml. of this solution (0.01 to 0.05 mg. of iron) into another 10 ml. volumetric flask and fill to the mark with regulating solution.* Use the zero point determination method in obtaining the polarogram.

After disconnecting the electrolysis system, place a part of the solution in the electrolysis cell. Start the polarograph, thus causing the light beam of the galvanometer to draw a zero line a few centimeters long upon the recording paper. Stop the motor and bring the contact to the potentiometer bridge back to the zero point. Connect the electrolysis system, start the polarograph, and record the iron wave from zero applied e.m.f. to the potential at which the limiting current is reached (not more than 60 mv.

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* See footnote, p. 361.

for the concentrations present in the samples). Use a galvanometer sensitivity of 1/30 to 1/50 depending on the concentration of the iron in the solution. Measure the wave height from the zero line obtained in the first step of the procedure. The solution to be tested should be in contact with the mercury only a short time before the recording is made, and the cell containing mercury and solution should not be shaken.

Analysis of Urine

Nickel and manganese can be determined separately in solutions of urine ash, but with urines which contain minute amounts of these elements, sufficient accuracy is obtained by the time-saving procedure of simultaneous determination of copper, zinc, nickel and manganese.

Copper, Zinc, Nickel, Manganese

In a platinum crucible, carefully evaporate the 50 ml. of ash solution (representing 250 to 300 ml. of fresh urine) to dryness. Dissolve the residue in 10 or 15 ml. of regulating solution,* and filter. Retain the precipitate for iron determination. Place the solution in the electrolytic cell and remove oxygen with nitrogen. Take the polarograms for copper and zinc in the same manner and with the same galvanometer sensitivities as described for feces ash solution. For the evaluation of the polarograms use calibration curves performed with pure solutions (p. 353). For the determination of nickel and manganese, leave the solution in the electrolytic cell and repeat the polarogram with a total potential drop of 3 volts across the bridge. Record the nickel wave from the zero line starting at about 0.7 volt total applied e.m.f. with sufficient galvanometer sensitivity. Record the manganese wave from the zero line of the recording paper shortly after the zinc wave is completed. Read the amounts of nickel and manganese on respective calibration curves.

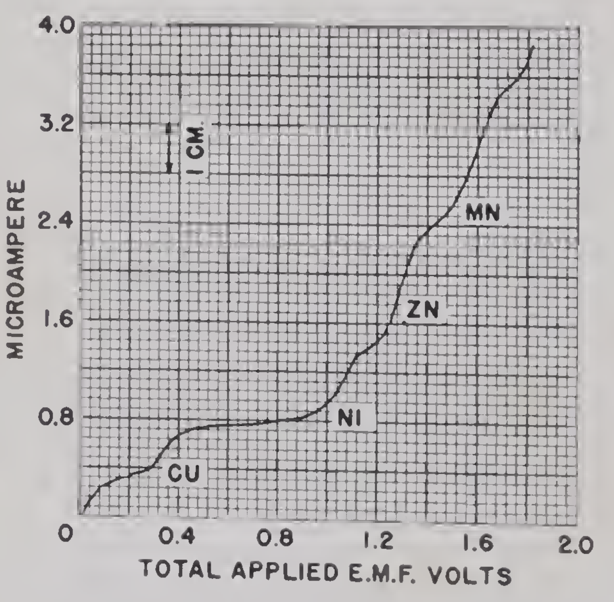

Figure 63. Polarogram of copper, nickel, zinc and manganese in a solution of urine ash.

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* Conc. NH_4Cl containing 5 gm. dextrin per liter.

COMPILATION AND TREATMENT OF DATA

Although the samples of food, urine and feces which are analyzed in a metabolic balance study may adequately represent the intake and outgo of a food constituent for a specific period of time, the analytical values are subject to the error inherent in the methods of analysis and calculation and are portrayed in the retentions.

The error of an analytical method is not limited to the accuracy of the method but is varied by the dexterity, competence and experience of the individual performing the analyses. In routine laboratory procedure it is customary to check each determination by making analyses upon triplicate samples. If the results of at least two of the replicates do not agree within the error permitted by the method, additional determinations are carried out until this requirement is fulfilled. In the work of the Laboratory "method errors" have been reduced to as much as one-half of the allowable error by reason of highly-trained and experienced personnel, *i.e.*, some methods with a normal accuracy of ±2 per cent have yielded results within ±1 per cent, under the conditions which existed during the studies of nutrition and chemical growth in childhood.

Frequently, several analyses upon comparative samples are required to obtain duplicate, "check" results. Common practice has been to discard all values which the chemist *felt* were invalidated by imperfections in procedure or their non-conformity with the other replicates. Such subjective estimations have a distinct bearing upon the compilation of final results, for only the chemist can observe the "deviations from normal" which occur during the chemical procedure. However, in recent years new statistical procedures have been developed which are of great assistance in determining, objectively, which values of a series should be eliminated.

In a metabolic balance study of 7 children during 11 consecutive, five-day balance periods, triplicate analyses were made upon representative samples of the food intake during each period and upon the corresponding feces and urine. The food composites and excreta samples were analyzed for nitrogen, carbon, calcium, magnesium, sodium, potassium, phosphorus, chlorine, sulfur, and their heat of combustion was determined. Food and feces were analyzed for fat, lignin, cellulose, and hemicellulose. The results of the individual analyses were calculated upon a daily basis and the accuracy of the values determined by application of the Analysis of Variance,[320] combined with the Chi-Square technique.

Precision of Chemical Methods

The Analysis of Variance technique, given in great detail by Snedecor, Lindquist,[202] and others, provides a method of segregating the variability traceable to specific sources and is applicable to small numbers of observations. By this analysis the total variability, commonly represented by the standard deviation, can be broken up into its various components. In handling data from metabolic studies, we have been able to separate the variability in replicate analyses from that caused by changes from period to period or from child to child.

Application of the Analysis of Variance to the results of metabolic balance determinations is best explained by illustration. The results of calcium determinations on 11 food composites, each representing a five-day period, is used as an example of the method of calculation. This is a relatively simple example, since the dietary was made up of constant quantities of the same foods. A more complicated Analysis of Variance problem results when differences in performance of individual subjects must be considered.

Application of Analysis of Variance and Chi-Square Test to Analyses of Food Composites

ORIGINAL DATA

Values in milligrams per day from triplicate analyses for calcium in food composites

Period	Samples			Greatest difference	Total
1	356	353	360	7	1069
2	350	355	361	11	1066
3	364	362	363	2	1089
4	337	333	341	24	1011
5	328	308	317	20	953
6	325	337	331	30	993
7	345	342	344	3	1031
8	348	345	354	9	1047
9	336	323	349	26	1008
10	353	349	352	4	1054
11	375	376	376	1	1127
Total					11492

Analysis of Variance

$$\text{Correction factor (C. F.)} = \frac{(\text{total sum})^2}{\text{number of samples}} = \frac{(11492)^2}{33} = 4002002$$

$$\frac{\begin{matrix}\text{Sum of squares}\\ \text{of period totals}\end{matrix}}{\text{No. samples per period}} - \text{C. F.} = \frac{(1069)^2 + (1066)^2 + \cdots + (1127)^2}{3} - 4002002$$

$$= 4008925 - 4002002 = 6923$$

$$\text{Sum of squares of each value} - \text{C. F.} = 356^2 + 350^2 + \cdots + 376^2 - 4002002$$

$$= 4010362 - 4002002 = 8360$$

	Degrees of freedom	Sum of squares	Mean squares	SD
Variation between periods	10	6923	692.30	26.3
Variation within periods	22	1437	65.32	8.1
Total variation	32	8360		

The standard deviation (SD) obtained between periods, 26.3 mg. of calcium per day, is more than three times as large as the standard deviation within periods, 8.1 mg. per day. The variation between periods may be attributable to weighing and preparation of daily samples and to food variability; the variation within the period may be due to mixing of the sample, weighing of aliquots and to errors inherent in the chemical methods.

Chi-Square Test

The chi-square test is represented by the formula:

$$\chi^2 = \frac{(\text{observed range} - \text{theoretical range})^2}{\text{theoretical range}}$$

It is commonly used as a means of comparing a series of figures with some standard or with another series as a measure of goodness of fit. Having obtained a series of values, we can, by means of this formula, compare them with a standard series and determine, by means of statistical tables* for the distribution of χ^2, how often one would expect to find a difference as great as existed between the two series.

If χ^2 is equal to zero, then the two series are identical. Referring to p. 27 of Fisher and Yates,[98] (or any other table giving the distribution of χ^2) we find the probability corresponding to $\chi^2 = .0157$ to be .99 when the number of degrees of freedom is one. This means that if χ^2 is found to be 0.0157, then we can expect to find a larger difference (observed−theoretical) in 99 out of a hundred cases, indicating the difference to be so small that the distributions are practically identical. However, for $\chi^2 = 10.827$ the probability is 0.001; and a larger difference would occur only once in a 1000 times.

This formula, can be transposed, however, enabling one to solve for the observed or theoretical value if an arbitrary value is assigned to χ^2. If we set out to find the range that would differ from a theoretical range by a value large enough to occur only once in a thousand times, then we would solve for the observed range in the following manner.

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* Fisher and Yates[98] have tabulated the probability of occurrence of various χ^2 values.

Let: Δ = observed range and KS = theoretical range

[K = mean range of samples of three in standard deviation units taken from normal population and S = standard deviation within periods]

Then: $$\chi^2 = \frac{(\Delta - KS)^2}{KS}$$

Solving for Δ:

$$\chi^2 KS = \Delta^2 - 2\Delta KS + K^2S^2$$

$$\Delta^2 - 2\Delta KS + K^2S^2 - \chi^2 KS = 0.$$

$$\Delta = \frac{2KS \pm \sqrt{4K^2S^2 - 4K^2S^2 + 4\chi^2 KS}}{2}$$

$$\Delta = \frac{2KS \pm \sqrt{4\chi^2 KS}}{2}$$

$$\Delta = KS \pm \sqrt{\chi^2 KS}$$

In the case of the calcium distribution we have previously calculated the standard deviation, $S = 8.1$ mg. per day, for the replicate analyses. According to Pearson's tables[261] the theoretical range for samples of three is $K = 1.69257$ expressed in standard deviation units. Therefore 1.69257 times 8.1 will give the theoretical range and χ^2 is arbitrarily taken as 10.827 for $P = 0.001$.

$$\Delta = 1.69257(8.1) \pm \sqrt{(10.827)(1.69257)(8.1)}$$

$$\Delta = 26$$

This observed range of 26 mg. of calcium per day indicates that under similar conditions of obtaining aliquots and applying this chemical procedure we would not expect to find a difference among replicate analyses to exceed 26 mg. in more than once out of a thousand cases. In the original data the triplicate values for period six had a greatest difference of 30, which is larger than the allowed difference of 26. If we discard the value 355 the difference becomes 12. The difference for period nine is exactly 26, but the values are equidistant, preventing a choice among them. The difference for period four is nearly 26; if we discard 357 the difference is 8.

By the use of the statistically determined, allowable difference between aliquots for the same period, the two values, 355 and 357, would be discarded as "freak" cases. After discarding the freak values the accuracy of the analytical method may be redetermined by the same technique, but to make the series of figures complete, values must be substituted for those discarded.

Revised correction factor for the original data = 3971415.

Substituting 337, the mean of 333 and 341, for 357 in period four and 331, the mean of 325 and 337, for 355 in period six, the series of values is again complete. (In applying the statistical treatment, substitution of the mean values in the series is recognized in the "degrees of freedom" allowed in the evaluation.)

	Degrees of freedom	Sum of squares	Mean squares	SD
Variation between periods	10	7817	781.7	28.0
Variation within periods	20	786	39.3	6.3
Total	30	8603		

$\Delta = 22$

The standard deviation within the periods is 6.3 mg. The greatest percentage difference one would expect in approximately two-thirds of the cases would be:

$$\frac{200\ \text{SD}}{\text{mean}} = \frac{200\ (6.3)}{347} = 3.6 \text{ per cent.}$$

Application of Analysis of Variance and Chi-Square Test to Analyses of Feces

Since individual variation is represented by the results of analyses of excretion, the details of the Analysis of Variance as applied to fecal excretion of sodium illustrates the more complicated application of the method.

ORIGINAL DATA

Values in milligrams per day from triplicate analyses for sodium in feces

CHILD I

Period	Samples			Greatest difference	Total
1	34	34	35	1	103
2	5	6	6	1	17
3	11	11	11	0	33
4	8	7	12	5	27
5	20	21	20	1	61
6	11	13	10	3	34
7	13	14	13	1	40
8	12	12	14	2	38
9	48	48	49	1	145
10	22	25	23	3	70
11	17	16	18	2	51
Total					619
CHILD II					
1	24	28	24	4	76
2	17	17	18	1	52
3	18	19	14	5	51
4	15	14	14	1	43
5	9	9	9	0	27
6	8	9	8	1	25
7	10	10	8	2	28
8	13	13	17	4	43
9	28	28	28	0	84
10	13	12	12	1	37
11	8	9	9	1	26
Total					492

CHILD III

1	17	17	17	0	51
2	25	22	25	3	72
3	14	16	16	2	46
4	8	8	10	2	26
5	31	33	33	2	97
6	6	6	6	0	18
7	13	13	12	1	38
8	12	11	11	1	34
9	43	43	44	1	130
10	17	18	19	2	54
11	7	7	6	1	20
Total					586

CHILD IV

1	77	80	79	3	236
2	31	27	28	4	86
3	16	16	16	0	48
4	12	13	13	1	38
5	30	29	31	2	90
6	26	26	26	0	78
7	17	18	17	1	52
8	23	23	22	1	68
9	17	16	16	1	49
10	35	33	34	2	102
11	11	11	12	1	34
Total					881

CHILD V

1	10	10	10	0	30
2	17	18	17	1	52
3	18	18	17	1	53
4	20	18	18	2	56
5	20	18	18	2	56
6	17	15	16	2	48
7	24	23	23	1	70
8	25	29	30	5	84
9	30	34	34	4	98
10	18	19	22	4	59
11	13	12	12	1	37
Total					643

CHILD VI

1	86	88	88	2	262
2	78	78	77	1	233
3	47	49	49	2	145
4	55	54	54	1	163
5	40	40	41	1	121
6	22	23	22	1	67
7	30	28	29	2	87
8	65	67	68	3	200
9	80	83	82	3	245
10	63	63	63	0	189
11	73	72	74	2	219
Total					1931

CHILD VII

1	33	33	33	0	99
2	18	19	17	2	54
3	17	19	16	3	52
4	12	12	11	1	35
5	14	15	11	4	40
6	25	22	25	3	72
7	18	18	18	0	54
8	34	34	34	0	102
9	36	30	35	6	101
10	23	23	24	1	70
11	62	62	61	1	185
Total					864

ALL CHILDREN

Period	All values	Period	All values
1	857	7	369
2	566	8	569
3	428	9	852
4	388	10	581
5	492	11	572
6	342		
Total			6016

Analysis of Variance

$$\text{Correction factor} = \frac{(\text{total sum})^2}{\text{number of samples}} = \frac{(6016)^2}{231} = 156676$$

$$\text{Total sum of squares} = (34)^2 + (5)^2 + (11)^2 + \cdots + (61)^2 - 156676$$

$$= 243230 - 156676 = 86554$$

$$\text{Sum of squares between periods} = \frac{(857)^2 + (566)^2 + \cdots + (572)^2}{21} - 156676$$

$$= 171340 - 156676 = 14664$$

$$\text{Sum of squares between children} = \frac{(619)^2 + (492)^2 + \cdots + (864)^2}{33} - 156676$$

$$= 201015 - 156676 = 44339$$

$$\text{Sum of squares, periods} \times \text{children} = \frac{(103)^2 + (17)^2 + \cdots + (185)^2}{3} - 156676 - 14664 - 44339$$

$$= 243009 - 156676 - 14664 - 44339 = 27330$$

Variation	Degrees of freedom	Sum of squares	Mean squares	SD
Between periods	10	14664	1466.40	38.3
Between children	6	44339	7389.83	86.0
Between periods × children	60	27330	455.50	21.3
Within periods	154	221	1.44	1.2
Total	230	86554		

$\Delta = KS \pm \sqrt{\chi^2 KS}$ (See derivation of formula with calcium in food, p. 366)

$\Delta = 1.69257(1.2) \pm \sqrt{10.827(1.69257)(1.2)}$

$\Delta = 7.$

The maximum theoretical difference to be expected among replicates 999 times out of 1000 is 7 mg. of fecal sodium. No observed difference in the series is as large as 7, therefore all values are retained and the accuracy of the analyses may be determined directly:

$$\frac{200\ \text{SD}}{\text{mean}} = \frac{200(1.2)}{26} = 9.2 \text{ per cent.}$$

In the calculation shown for fecal sodium, the standard deviation betwen periods of 38.3 mg. of fecal sodium per day is more than thirty times as large as the standard deviation within periods of 1.2 mg. per day. The variability between periods may, in part, be due to errors in separation, weighing, or mixing of feces, whereas the variation within periods is due to measuring of aliquots and errors inherent in the sodium method. The variations occurring from period to period and child to child are larger and more significant then the variability resulting from errors inherent in the chemical procedure.

The results of application of the Analysis of Variance and Chi-Square technique to other elements are presented in Table 122.

TABLE 122

VARIABILITY AND MAXIMUM EXPERIMENTAL DEVIATION ALLOWABLE IN CHEMICAL ANALYSES AS DETERMINED BY ANALYSIS OF VARIANCE AND CHI-SQUARE TECHNIQUE*

	VARIABILITY OF DAILY VALUES			MAXIMUM ALLOWABLE DEVIATION		
	Food composite	Feces	Urine	Food composite	Feces	Urine
				per cent	*per cent*	*per cent*
Nitrogen (gm.)	0.06	0.17	0.43	0.3	0.8	0.4
Calcium (mg.)	22	14	11	3.6	1.1	3.9
Magnesium (mg.)	16	9	9	3.8	2.2	3.5
Sodium (mg.)	29	7	32	1.0	9.2	1.2
Potassium (mg.)	41	11	41	1.1	1.5	1.1
Phosphorus (mg.)	95	11	12	2.4	1.1	0.9
Chlorine (mg.)	18	4	28	1.4	6.4	0.7
Sulfur (mg.)	22	7	16	2.6	3.2	1.7
Fat (gm.)	0.52	0.17		1.6	4.3	
Heat of combustion (Cal.)	17	6	6	0.1	2.7	4.1

* Magnitudes of the daily averages are shown in the tables in Chapter VIII.

The greatest differences that would occur between samples 999 times in a 1000 are listed, as well as the maximum per cent difference from the mean that would occur in two-thirds of the cases. These figures indicate the greatest variation that could be expected; our analyses show much less variation than would be allowable, statistically. The values found, by statistical procedure, to be freak cases that should be discarded were the same values which had been eliminated by the chemists.

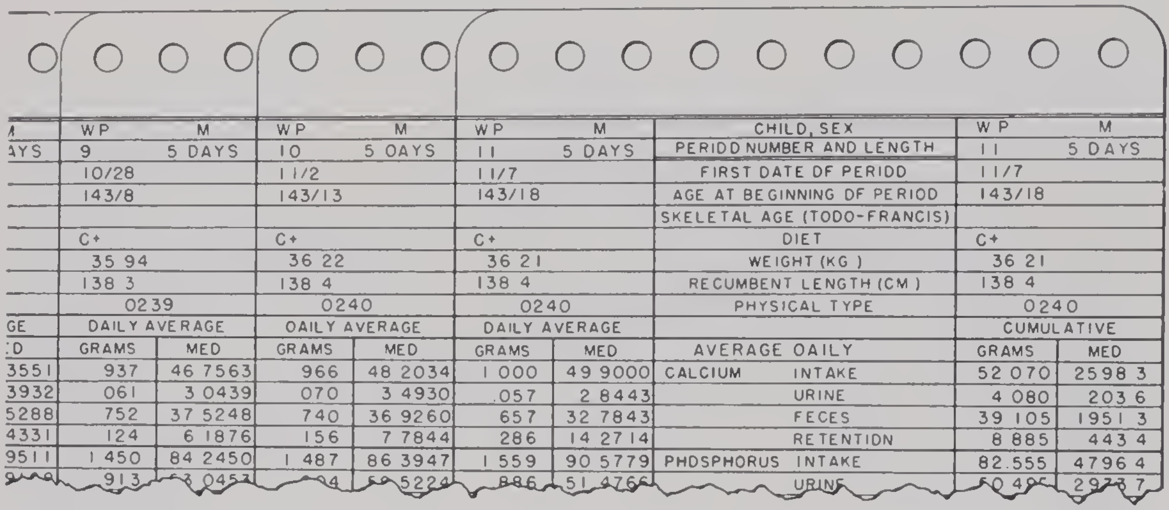
COMPILATION OF DATA

All original data from the studies of nutrition and chemical growth in childhood were thoroughly checked by staff members of the Laboratory. Each final value was checked by at least two individuals who were experienced in the chemical procedure by which the result was obtained, and all calculations were rechecked by two members of the statistical staff, using different calculating machines. Results of replicate analyses are frequently obtained in figures which imply an accuracy unjustified by the conditions of the entire study. In metabolic balance determinations the criteria for significance of the final values are largely subjective, based upon knowledge of the performance of the human body and the conditions under which the data were obtained. We have found it wise, however, to record replicate figures to the limits of estimation and average the full values to obtain our final results. Accuracy in interpretation can be further enhanced by using the complete final results of all determinations in making calculations with the data, "rounding" the figures to the last digit considered significant only after the tabulation has been made.

Accumulation of several hundred thousand original data does not complete an investigation. Assembly and interpretation of the values require a greater outlay of time than procurement of the original figures. In this work three and four electric machines have been continuously used for more than five years to calculate the final values. With a project devised to obtain specific information involving group averages and interrelationships, a punch card system adequately serves in recapitulating the data, for all variables to be related can be recorded on the same card. Every mathematical procedure which would be necessary could not be foreseen in the studies of nutrition and chemical growth in childhood and since the variables to be recorded were too numerous to be included on one punch card, this system was inadequate.

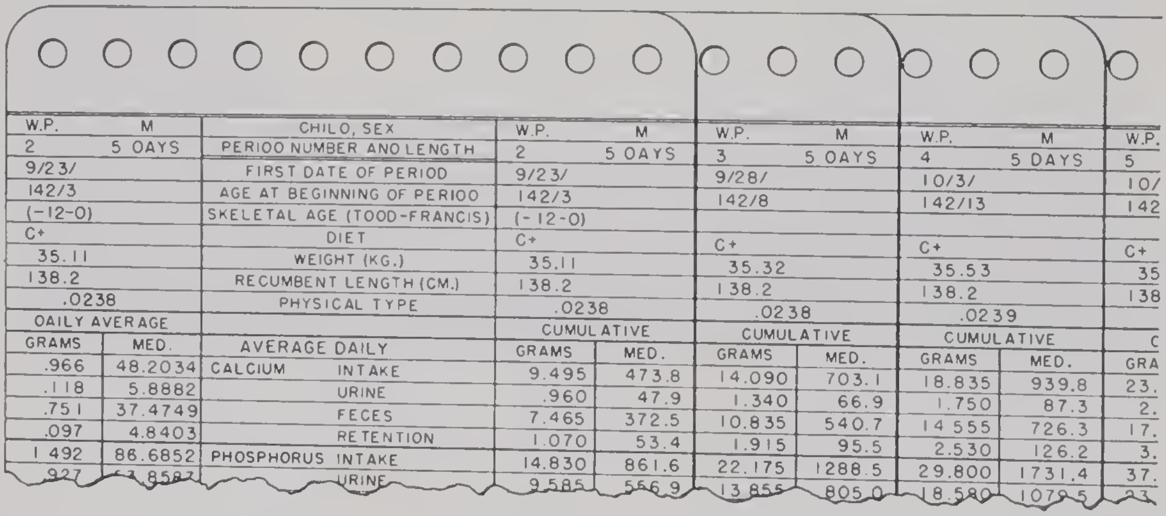
With the cooperation of the manufacturer, the "McBee Multi-spred" system for strip accounting* was adapted to the recording of the values.

All data obtained for a single individual during one balance period were recorded upon one of the strip sheets, as indicated in Figures 64 and 65. In the two columns at the left side of the sheet, average daily values for the period are given in grams and milliequivalents; in the two columns at the right, the values for the period are cumulated with the corresponding values for all preceding periods. In Figure 64, the left columns of the upper portion of the sheet for W. P. during the eleventh period of a study show the child's average daily intake (1.000 gm. or 49.9000 meq.), outgo in urine (0.057 gm. or 2.8443 meq.) and feces (0.657



	WP	M	WP	M	WP	M	CHILD, SEX	W P	M
AYS	9	5 DAYS	10	5 OAYS	11	5 DAYS	PERIDD NUMBER AND LENGTH	11	5 DAYS
	10/28		11/2		11/7		FIRST DATE DF PERIDD	11/7	
	143/8		143/13		143/18		AGE AT BEGINNING DF PERIOD	143/18	
							SKELETAL AGE (TODO-FRANCIS)		
	C+		C+		C+		DIET	C+	
	35 94		36 22		36 21		WEIGHT (KG)	36 21	
	138 3		138 4		138 4		RECUMBENT LENGTH (CM)	138 4	
	0239		0240		0240		PHYSICAL TYPE	0240	
GE	DAILY AVERAGE		OAILY AVERAGE		DAILY AVERAGE			CUMULATIVE	
:D	GRAMS	MED	GRAMS	MED	GRAMS	MED	AVERAGE OAILY	GRAMS	MED
3551	937	46 7563	966	48 2034	1 000	49 9000	CALCIUM INTAKE	52 070	2598 3
3932	061	3 0439	070	3 4930	.057	2 8443	URINE	4 080	203 6
5288	752	37 5248	740	36 9260	657	32 7843	FECES	39 105	1951 3
4331	124	6 1876	156	7 7844	286	14 2714	RETENTIDN	8 885	443 4
9511	1 450	84 2450	1 487	86 3947	1 559	90 5779	PHDSPHORUS INTAKE	82.555	4796 4
[illegible]	913	[illegible]	[illegible]	[illegible]	886	51 476	URINE	[illegible]	[illegible]

FIGURE 64. Upper portion of strip sheets arranged for use of average daily values.



W.P.	M	CHILO, SEX	W.P.	M	W.P.	M	W.P.	M	W.P.
2	5 OAYS	PERIOO NUMBER ANO LENGTH	2	5 OAYS	3	5 OAYS	4	5 DAYS	5
9/23/		FIRST DATE OF PERIOD	9/23/		9/28/		10/3/		10/
142/3		AGE AT BEGINNING OF PERIOO	142/3		142/8		142/13		142
(-12-0)		SKELETAL AGE (TOOD-FRANCIS)	(-12-0)						
C+		DIET	C+		C+		C+		C+
35.11		WEIGHT (KG.)	35.11		35.32		35.53		35
138.2		RECUMBENT LENGTH (CM.)	138.2		138.2		138.2		138
.0238		PHYSICAL TYPE	.0238		.0238		.0239		
OAILY AVERAGE			CUMULATIVE		CUMULATIVE		CUMULATIVE		C
GRAMS	MED.	AVERAGE DAILY	GRAMS	MED.	GRAMS	MED.	GRAMS	MED.	GRA
.966	48.2034	CALCIUM INTAKE	9.495	473.8	14.090	703.1	18.835	939.8	23.
.118	5.8882	URINE	.960	47.9	1.340	66.9	1.750	87.3	2.
.751	37.4749	FECES	7.465	372.5	10.835	540.7	14 555	726.3	17.
.097	4.8403	RETENTION	1.070	53.4	1.915	95.5	2.530	126.2	3.
1 492	86.6852	PHOSPHORUS INTAKE	14.830	861.6	22.175	1288.5	29.800	1731.4	37.
.927	[illegible]	URINE	9.585	566.9	13.855	805.0	18.580	1079.5	[illegible]

FIGURE 65. Upper portions of strip sheets arranged for use of cumulative values.

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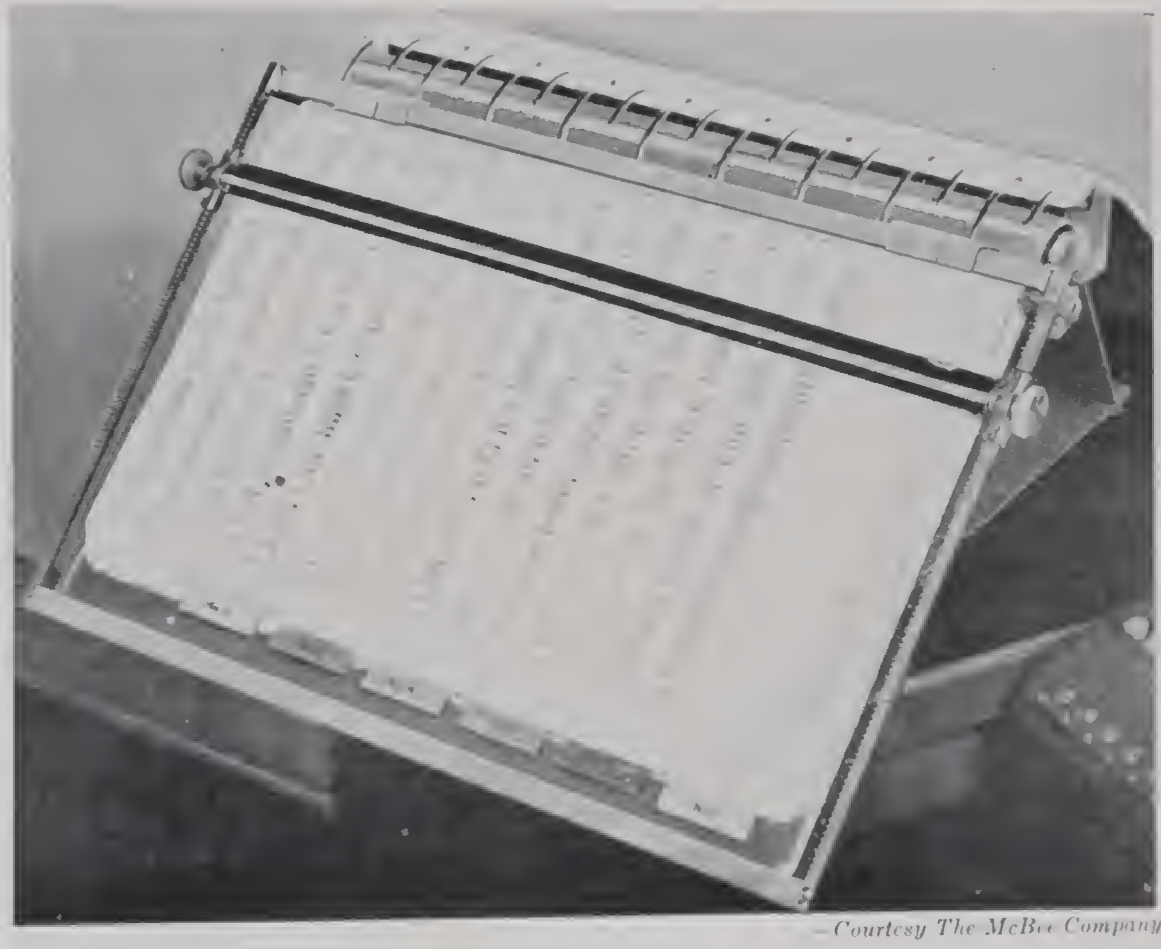
* The McBee Company, Athens, Ohio.

gm. or 32.7843 meq.) and retention (0.286 gm. or 14.2714 meq.) of calcium. The right columns of the same sheet show the calcium balance data accumulated during the 55 days in the eleven periods, in both grams and milliequivalents. After the recorded values have been used in calculating, the resulting figure is rounded to the number of decimal places considered significant.

The strip sheets may be arranged (see Figures 64 and 65) in the large, stiff-cover binder shown in Figure 66 so that either the average daily or cumulative figures for consecutive periods can be compared directly without interference by the other two columns of figures or the center headings of sheets following the first or preceding the last of a spread. The individual records of each child for a single period may also be arranged in the binder so that the same periods for a number of children can be compared, or so that the sheets may be compared on the basis of chronological age, skeletal age, diet, weight, recumbent length, or physical type; or the intake, outgo in urine and feces, or retention of any element.



—*Courtesy The McBee Company*

FIGURE 66. Stiff-cover binder for strip sheets used to record final data.

Data which were not obtained upon the basis of metabolic balance periods (daily nitrogen and sulfur partitions of urine, blood analyses and examination records) were recorded upon single sheets of dimensions equal to the entire spread of the binder and punched to allow insertion in it. All of the final record sheets were typed in triplicate and all calculations and transfers of figures were checked against the original data sheets by at least two trained staff members, using different calculating machines, to eliminate personal or mechanical errors.

The data presented in this volume have not been subjected to a complete statistical exploration; however, some statistical procedures have been applied in the interest of accuracy. The details of the application of the statistical procedures to the data in Volume I of *Nutrition and Chemical Growth in Childhood* are given on the following pages, but for full information on the development and other uses of the methods, the reader should refer to special treatises on the subject.

Measures of Central Tendency and Variability

When dealing with a mass of figures it becomes necessary to describe them in a form which is condensed and easily understood. Usually the values group themselves around some central point. The arithmetic mean, usually termed the mean, is a measure of this central tendency and is the point above and below which the sums of the deviations are equal. The variability, or dispersion, of these values around the mean is described by the standard deviation. These measures of central tendency and variability, the mean and standard deviation, are used throughout the present volume. The mean is the sum of the observed values divided by the number of observations. The formula for the standard deviation using $(N-1)$ was chosen in preference to the one using N because of the size of the samples.

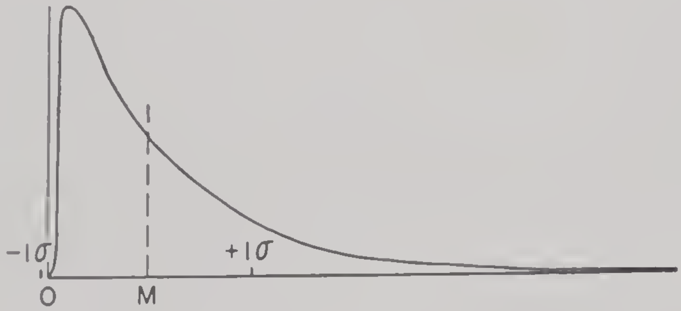
$$SD = \sqrt{\frac{\Sigma x^2 - N\bar{x}^2}{N-1}}$$

When the Σx^2 = the sum of the squares of the observations
N = the number of observations
$\bar{x}^2$ = the square of the mean

In averaging metabolic balance data every precaution should be observed to avoid improper "weighting." Final balance data are usually placed upon a daily basis and represent the average values for each day of a specific balance period. If a number of consecutive balances are determined for the same individual and all of the balance periods are of the same length, then an average of the average daily values represents the mean for the individual over the entire time; however, if the balance periods are of different lengths the true mean should be calculated, multiplying each average daily figure by the number of days in that period and dividing the sum of all these values by the total number of days. The averages given in this volume are based on all the observations for the group. The averages for the age-groups are not weighted for children nor are the total averages weighted for age or child. We have grouped all our available data regardless of the number of observations made on any one child or age group.

The standard deviation (SD) provides a measure of the spread. The greater the range of points in a group of data the larger the standard deviation (except in cases of extreme skewness). In a normal distribution, 99.73 per cent of the cases fall within ±3 SD from the mean, 99.99366 per cent fall within ±4 SD. For example, if the mean of a normal distribution is 20 and the standard deviation is 2, then 99.73 per cent of the cases lie between 14 and 26.

Occasionally a standard deviation is larger than the mean, indicating that negative values are involved (which does not preclude normal distribution) or that the frequency distribution is positively skewed in a manner similar to



in which there is a "piling up" of values at the lower end of the distribution curve and only a few values higher up on the curve.

The standard error of the mean, the standard deviation divided by the square root of the number of cases, is a measure of the reliability of the mean, *i.e.*, a measure of the scatter one would find if they were to plot the means of other similar samples.

Percentages and Ratios

The percentage figures in the text are percentages determined by dividing averages; such as average urine excretion, by average intake. This applies to the values given as the per cent of intake excreted in the urine or feces, the per cent of intake retained and the per cent of total excretion appearing in the urine and the feces. These percentages are equivalent, theoretically, to those obtained for the entire period of study. They are not averages of the percentages determined per period. The two methods of calculating percentage of intake excreted in urine or feces, or retained, and the percentage of outgo excreted in urine or feces, may provide very different values, for the average of the percentages per period may be unduly influenced by a freak case.

The ratios given in this volume also are calculated from final average values, for example, the average calcium intake divided by the average phosphorus intake values over an entire investigation have been presented, rather than the average of the ratios for individual periods.

When per cent difference values are given, the point of reference is very important and should be considered in interpreting the results. For example, in comparing analyses given by two investigators, A and B, if percentage differences are determined for the values of A with respect to those of B, the negative differences determined for A are minimized and the positive differences exaggerated, but if differences for A are determined, with respect to the values of *A* the reverse is true. In the following data the difference between the values of A and B are equivalent in amount but the several percentages are dissimilar.

Results obtained by			Per cent difference of A's results with respect to results obtained by	
A	B	A in terms of B	A	B
200	100	100 >	50 >	100 >
100	200	100 <	100 <	50 <

Tests of Significance of Difference

If, for a large number of pairs of observations, the difference, however small, is consistently in one direction we conclude that a real difference exists between the two series of observations. For a small number of paired values we are limited to inspection in attempting to discern trends, but for sufficiently large collections of data statistical methods based on the laws of probability have been devised to test the significance of differences between comparable series of values. These tests, some finer instruments than others, determine whether the observed differences could exist between samples drawn from a homogeneous population. If the difference is greater than could possibly be accounted for by chance, in 999 out of 1000 times, then the conclusion is that the difference, at least in part, is a true difference and statistically significant. If the difference is less than *could be* accounted for by chance, then it is not known if there is a *real* difference because it is possible to account for the entire difference by chance.

Critical ratio: The critical ratio, a method of testing the significance of the difference between two groups, is equal to

$$(M_1 - M_2)/\sqrt{\mathrm{SE}_{m_1}^2 + \mathrm{SE}_{m_2}^2}.$$

where M_1 and M_2 are the means of the two groups, respectively, and SE_{m1} and SE_{m2} are the standard errors of the means. When the critical ratio is 3.3 or more, then in 999 cases out of 1000 there is a true difference between the groups, for the total difference cannot be entirely accounted for by chance. When the critical ratio is less than 3.3, the difference may or may not be a real difference, for it is possible that the entire difference is accounted for by chance.

Analysis of Variance: The Analysis of Variance technique is a finer instrument than the critical ratio, for it enables segregation of the variations traceable to specific sources. The method affords a basis of judging whether we are dealing with samples from a homogeneous population. If the observed ratio is greater than the theoretical ratio (determined by R. A. Fisher and tabulated by Snedecor[320]) the conclusion is that the difference, at least in part, is a true difference. Again, if the observed ratio is less than the theoretical ratio, the method does not separate the *true difference*, which may or may not exist, from that accounted for by chance. The method is valuable in determining whether changes in retention, consequent to changes in intake, are significant; and to compare results obtained by various methods of analysis, for instance, polarograph *vs.* chemical.

Measures of Relationship

The Pearson Product Moment Correlation Coefficient is a measure of the relationship of two variables. The resulting figure is within the range -1 to $+1$. A positive correlation coefficient indicates that if one variable is high the other is also high; a negative figure indicates that if one variable is high the other must be low. A perfect prediction is possible but extremely rare. A zero correlation coefficient indicates no relationship.

The correlation coefficient is calculated by the formula:

$$r_{xy} = \frac{N\Sigma xy - \Sigma x\Sigma y}{\sqrt{[N\Sigma x^2 - (\Sigma x)^2][N\Sigma y^2 - (\Sigma y)^2]}}\,.$$

In which: N = the number of observations
Σx = sum of the observations for one variable
Σy = sum of the observations for the other variable
Σx^2 = sum of the squares of the first variable
Σy^2 = sum of the squares of the second variable
$(\Sigma x)^2$ = the square of the sum of the first variable
$(\Sigma y)^2$ = the square of the sum of the second variable
Σxy = the sum of the cross products of the two variables

The standard error indicates the reliability of the correlation coefficient.

$$\text{Standard Error} = \text{SE}_r = \frac{(1 - r^2)}{\sqrt{N}}\,.$$

The number of observations must be taken into consideration. A high correlation coefficient is not necessarily a reliable figure if based on few data. A lower correlation coefficient based on thousands of cases would be more reliable. The reliability of the relationship can only be judged when both the correlation coefficient and the standard error of the coefficient are known. For example, $r \pm \text{SE}_r = .60 \pm .02$ indicates a reliable relationship, while $.60 \pm .40$ indicates an uncertain relationship.

Care must be exercised in interpreting correlation coefficients, for high correlations may be the result of characteristics inherent in the data. Sometimes, it is possible to predict the extent of a relationship from the nature of the variables. Our calculated absorption values [intake minus fecal excretion] provide a good example of this, when the fecal excretion is nearly constant. During 593 five-day balance periods the nitrogen intakes of 29 children averaged 10.64 ± 1.47 gm. per day and the nitrogen excretion in feces averaged 1.12 ± 0.18 gm. per day. A standard deviation of 0.18 indicates that the fecal excretion of 1.12 gm. is a relatively constant figure. Merely from observation of the figures involved we would expect a high correlation coefficient between intake and absorption; actually it is (.9924 ± .0006).

Knowing the correlation coefficient, one variable may be predicted from the other. The equation,

> $y = r_{xy}\mathrm{SD}y(x-\bar{x})/\mathrm{SD}_x+\bar{y}$ where x and y are the variables, $\bar{x}$ and $\bar{y}$ are the means, and the SD_x and SD_y are the standard deviations,

enables the determination of y from a known x. If all the points were plotted on a scattergram this would be the equation of the best fitting straight line through the points drawn with respect to the x-axis. The y value predicted is the point on the line corresponding to the x-value.

The Standard Error of Estimate

$$\mathrm{SE}_{\mathrm{est}.y} = \mathrm{SD}_y\sqrt{1-r^2_{xy}}$$

gives an indication of the accuracy of the prediction. It is, in fact, the average SD for the columns of y's corresponding to their x-values. If r is equal to 1 or -1 the $\mathrm{SE}_{\mathrm{est}.y} = 0$ and the prediction is perfect; every point lies on a straight line as there is no scatter. A zero correlation coefficient gives a $\mathrm{SE}_{\mathrm{est}.y} = \mathrm{SD}_y$. When this is the case the relationship is of no value in prediction. The best prediction of y from any x would be the average of the y's.

When two variables are highly correlated one cannot conclude that one variable is dependent on the other or that one is caused by the other. It may be that both are the result of a third variable. For example, one may find a high relationship between height and body weight for a given group, which may consist of children of all ages. The correlation would be unusually high due to relationships between age and height and age and weight. A relation between height and weight could be worked out for children of the same age but this would require a large number of children and a great deal of work. An easier way to approach such a problem is by the use of partial correlation coefficients,

$$r_{01.234\cdots n} = \frac{r_{01.234\cdots(n-1)} - r_{0n.234\cdots(n-1)}r_{1n.234\cdots(n-1)}}{\sqrt{(1-r^2_{0n.234\cdots(n-1)})(1-r^2_{1n.234\cdots(n-1)})}}$$

in which the relationship between variable 0 and 1 may be determined, holding constant as many other variables as desired.

For this group of children from four to twelve years of age the zero-order coefficient of correlation for height and weight is 0.95 ± 0.01 whereas the partial correlation coefficient of height and weight, keeping age constant, is 0.73 ± 0.03, demonstrating that the high coefficient found originally was especially high because of the group involved, and that if the group had been homogeneous with respect to age the relationship would have been much lower.

It is sometimes desirable to determine the relationship between one variable and the best combination of a group of variables. In determining the possiblity of predicting a variable such as normal weight from a series of other measurements such as age, height, chest measurements, hip width, elbow and knee breadths, and any others that are known to show a relationship, the multiple correlation coefficient can be obtained:

$$R_{0.12345\cdots n} = \sqrt{1-(1-r^2{}_{0.1})(1-r^2{}_{02.1})(1-r^2{}_{03.12})(1-r^2{}_{04.123})\cdots(1-r^2{}_{0n.123\cdots n-1})}.$$

In the case of basal heat production and its relationship to body size

Basal *vs.* Age = 0.78 ± 0.03
Basal *vs.* Height = 0.79 ± 0.02
Basal *vs.* Weight = 0.79 ± 0.02
Basal *vs.* Surface area as determined by the DuBois Formula = 0.80 ± 0.02

The multiple correlation coefficient of basal heat production holding constant:

Age, height = 0.80 ± 0.02
Age, weight = 0.80 ± 0.02
Age, surface area = 0.81 ± 0.02
Age, height, weight = 0.81 ± 0.02
Age, height, weight, surface area = 0.92 ± 0.01

Basal heat production is correlated almost equally with age and measures of body size. By combining various measurements the correlation coefficient may be increased, thus permitting a better prediction with four rather than only one variable.

REFERENCES CITED

1. Albanese, Anthony A., L. Emmett Holt, Jr., Joseph E. Brumback, Jr., Marjorie Hayes, Charlotte Kajdi, and Dorothy M. Wangerin. Nitrogen balance in experimental lysine deficiency in man. *Proc. Soc. Exper. Biol. Med.*, 48: 728, 1941.
2. Ascham, Leah, Mary Speirs, and Dorothy Maddox. The availability of iron in various foods. *J. Nutrition*, 16: 425, 1938.
3. Babcock, S. M. Metabolic Water: Its Production and Role in Vital Phenomena. *Univ. Wis. Agr. Exper. Sta. Res. Bull.*, No. 22, 1912.
4. Barcroft, Joseph. The *Raison D'etre* of the Red Corpuscle. The Harvey Lectures Series, 17: 146, 1921. J. B. Lippincott Co., Philadelphia, 1923.
5. Bassett, Samuel H., and Helen E. Van Alstine. Mineral exchanges of man. IV. Variations in the mineral content of diets with a constant raw weight formula. *J. Nutrition*, 9: 175, 1935.
6. Bauer, Walter, Joseph C. Aub, and Fuller Albright. Studies of calcium and phosphorus metabolism. V. A study of the bone trabeculae as a readily available reserve supply of calcium. *J. Exper. Med.*, 49: 145, 1929.
7. Beach, Eliot F., Betty Nims Erickson, Samuel S. Bernstein, Harold H. Williams, and Icie G. Macy. The amino acid composition of erythrocyte posthemolytic residue of five mammalian species. *J. Biol. Chem.*, 128: 339, 1939.
8. Beach, Eliot F., Samuel S. Bernstein, Frances C. Hummel, Harold H. Williams, and Icie G. Macy. Total sulfur, cystine, and methionine content of blood globins of five mammalian species. *J. Biol. Chem.*, 130: 115, 1939.
9. Beach, Eliot F., Samuel S. Bernstein, Olive D. Hoffman, D. Maxwell Teague, and Icie G. Macy. Distribution of nitrogen and protein amino acids in human and in cow's milk. *J. Biol. Chem.*, 139: 57, 1941.
10. Beach, Eliot F., Samuel S. Bernstein, and Icie G. Macy. Intake of amino acids by breast-milk-fed infants and amino acid composition of cow's milk and human milk. *J. Pediatrics*, 19: 190, 1941.
11. Benedict, Francis G., and Fritz B. Talbot. Metabolism and Growth from Birth to Puberty. Carnegie Institution of Washington, Publication No. 302, 1921.
12. Benedict, Francis G. Vital Energetics. A Study in Comparative Basal Metabolism. Carnegie Institution of Washington, Publication No. 503, 1938.
13. Benedict, Stanley R. The estimation of total sulfur in urine. *J. Biol. Chem.*, 6: 363, 1909.
14. Benedict, Stanley R. Studies in creatine and creatinine metabolism. II. The estimation of creatine. *J. Biol. Chem.*, 18: 191, 1914.
15. Benedict, Stanley R., and Elizabeth Franke. A method for the direct determination of uric acid in urine. *J. Biol. Chem.*, 52: 387, 1922.
16. Bernstein, Samuel S., Robert L. Jones, Betty Nims Erickson, Harold H. Williams, Ira Avrin, and Icie G. Macy. A method for the preparation of posthemolytic residue or stroma of erythrocytes. *J. Biol. Chem.*, 122: 507, 1937.
17. Bernstein, Samuel S., Harold H. Williams, Frances C. Hummel, Marion L. Shepherd, and Betty Nims Erickson. Metabolic observations on a child with essential hyperlipemia. *J. Pediatrics*, 14: 570, 1939.
18. Bishop, Edna R. The calcium and phosphorus content of some Alabama vegetables. *J. Nutrition*, 8: 239, 1934.

19. Blazso, Sandor. Zum schwefel- und eiweissstoffwechsel des natürlich und künstlich ernährten säuglings. Ein beitrag zum Ernährungsproblem. *Ann. Paediät.*, 152: 302, 1939.

20. Block, Richard J. Comparative Biochemistry of the Proteins. Cold Spring Harbor Symposia on Quantitative Biology, 6: 79, 1938. The Darwin Press, New Bedford, Mass.

21. Bloor, W. R. Distribution of unsaturated fatty acids in tissues. III. Vital organs of beef. *J. Biol. Chem.*, 80: 443, 1928.

22. Bloor, W. R., Ruth Okey, and George W. Corner. The relation of the lipids to physiological activity. I. The changes in the lipid content of the corpus luteum of the sow. *J. Biol. Chem.*, 86: 291, 1930.

23. Bloor, W. R., and Ruth H. Snider. Phospholipid content and activity in muscle. *J. Biol. Chem.*, 107: 459, 1934.

24. Bloor, W. R. A Text Book of Biochemistry, Chapter 21. *Edited by* Benjamin Harrow and Carl P. Sherwin. W. B. Saunders Co., Philadelphia, 1935.

25. Boothby, Walter M., and Irene Sandiford. Normal values of basal or standard metabolism. A modification of the DuBois standards. *Am. J. Physiol.*, 90: 290, 1929.

26. Borgen, D. R., and C. A. Elvehjem. Factors affecting the determination of inorganic iron in animal tissues. *J. Biol. Chem.*, 119: 725, 1937.

27. Borsook, Henry, and Jacob W. Dubnoff. The formation of creatine from glycocyamine in the liver. *J. Biol. Chem.*, 132: 559, 1940.

28. Bosworth, A. W., H. I. Bowditch, and Louise A. Giblin. Studies of infant feeding. X. The digestion and absorption of fats. I. Calcium in its relation to the absorption of fatty acids. *Am. J. Dis. Child.*, 15: 397, 1918.

29. Bouslog, John S., T. D. Cunningham, James P. Hanner, James B. Walton, and Harold D. Waltz. Roentgenologic studies of the infant's gastrointestinal tract. *J. Pediatrics*, 6: 234, 1935.

30. Boyd, Edith. The experimental error inherent in measuring the growing human body. *Am. J. Phys. Anthrop.*, 13: 389, 1929.

31. Boyd, Eldon M. The lipid composition of the white blood cells in leukemia. *Arch. Path.*, 21: 739, 1936.

32. Boyd, Eldon M. The oxidative micro-estimation of blood lipids. *Am. J. Clin. Path.*, 8: 77, 1938.

33. Boyd, Oscar F., Carlos L. Crum, and J. F. Lyman. The absorption of calcium soaps and the relation of dietary fat to calcium utilization in the white rat. *J. Biol. Chem.*, 95: 29, 1932.

34. Brand, Florence C., and Warren M. Sperry. The determination of cerebrosides. *J. Biol. Chem.*, 141: 545, 1941.

35. Brdicka, R. Polarographic studies with the dropping mercury kathode. Part XXXI. A new test for proteins in the presence of cobalt salts in ammoniacal solutions of ammonium chloride. *Coll. Czech. Chem. Commun.*, 5: 112, 1933.

36. Brdicka, R. Polarographic studies with the dropping mercury kathode. Part XXXII. Activation of hydrogen in sulphydryl group of some thioacids in cobalt salt solutions. *Coll. Czech. Chem. Commun.*, 5: 148, 1933.

37. Breiter, Herta, Rosalind Mills, Julia Dwight, Beula McKey, Williamina Armstrong, and Julia Outhouse. The utilization of the calcium of milk by adults. *J. Nutrition*, 21: 351, 1941.

38. Brody, S., and R. C. Procter. Part V. Analyses of age changes in basal metabolism per unit weight in humans and pigeons with special reference to senescence. *In*, Growth and Development. With Special Reference to Domestic Animals. XXIV. The decline in energy metabolism per unit weight with increasing age in farm animals, laboratory animals, and humans. *Univ. Mo. Agric. Exper. Sta. Res. Bull.*, No. 176, 1932.

39. Brody, Samuel, Robert C. Procter, and Ural S. Ashworth. Growth and Development. With Special Reference to Domestic Animals. XXXIV. Basal metabolism, endogenous nitrogen, creatinine and neutral sulphur excretions as functions of body weight. *Univ. Mo. Agric. Exp. Sta. Res. Bull.*, No. 220, 1934.

40. Brody, Samuel. Relativity of physiologic time and physiologic weight. *Growth*, 1: 60, 1937.

40*a*. Bunting, Russell W. Recent developments in the study of dental caries. *Science*, 78: 419, 1933.

41. Burroughs, E. Wise, Helen S. Burroughs, and H. H. Mitchell. The interdependence among amino acids in their utilization in the endogenous metabolism. *J. Nutrition*, 19: 385, 1940.
42. Butler, A. M., and Elizabeth Tuthill. An application of the uranyl zinc acetate method for determination of sodium in biological material. *J. Biol. Chem.*, 93: 171, 1931.
43. Butler, Allan M., and Eaton M. MacKay. The influence of the sodium and potassium content of the diet upon the sodium concentration of human centrifuged red blood cells. *J. Biol. Chem.*, 106: 107, 1934.
44. Campbell, H. L., Otto A. Bessey, and H. C. Sherman. Adult rats of low calcium content. *J. Biol. Chem.*, 110: 703, 1935.
45. Cannon, Walter B. Bodily Changes in Pain, Hunger, Fear and Rage. An Account of Recent Researches into the Function of Emotional Excitement. Second edition. D. Appleton-Century Co., New York, 1936.
46. Cannon, Walter B. Digestion and Health. W. W. Norton and Co., Inc., New York, 1936.
47. Chargaff, Erwin, Fredric W. Bancroft, and Margaret Stanley-Brown. Studies on the chemistry of blood coagulation. III. The chemical constituents of blood platelets and their rôle in blood clotting, with remarks on the activation of clotting by lipids. *J. Biol. Chem.*, 116: 237, 1936.
48. Clark, Guy W. Studies in the mineral metabolism of adult man. *Univ. Calif. Publ. in Physiol.*, 5: 195, 1926.
49. Clark, W. Mansfield. The Determination of Hydrogen Ions. An Elementary Treatise on Electrode, Indicator and Supplementary Methods. With an Indexed Bibliography on Applications. Third edition. The Williams and Wilkins Company, Baltimore, 1928.
50. Cohen, Eugene and C. A. Elvehjem. The relation of iron and copper to the cytochrome and oxidase content of animal tissues. *J. Biol. Chem.*, 107: 97, 1934.
51. Coleman, J. M., and R. W. Ruprecht. The effect of fertilizers and soil types on the mineral composition of vegetables. *J. Nutrition*, 9: 51, 1935.
52. Committee on Foods and Nutrition, National Research Council. Planning Diets by the New Yardstick of Good Nutrition. Bur. Home Econ., U. S. Dept. Agric., July, 1941. [*J.A.M.A.*, 116: 2601, 1941.]
53. Cooley, Thomas B., Grover C. Penberthy, Lillian Armstrong, Helen A. Hunscher, Frances Cope, and Icie G. Macy. Mineral metabolism. A case of osteopsathyrosis and one of ununited fracture. *Am. J. Dis. Child.*, 50: 431, 1935.

53*a*. Cori, Carl T. *In* A Symposium on Respiratory Enzymes. Univ. Wisconsin Press, May, 1942.

54. Cowgill, George R., and William E. Anderson. Laxative effects of wheat bran and "washed bran" in healthy men. A comparative study. *J.A.M.A.* 98: 1866, 1932.
55. Cowgill, George R., and Albert I. Sullivan. Further studies on the use of wheat bran as a laxative. Observations on patients. *J.A.M.A.*, 100: 795, 1933.
56. Cowgill, George R. The Vitamin B Requirement of Man. Yale University Press, New Haven, 1934.
57. Cozzi, Danilo. The determination of ascorbic acid in fruit juices. *Ann. chim. applicata*, 29: 434, 1939.
58. Cuthbertson, David Paton, Alexander McCutcheon, and Hamish Nisbet Munro. A study of the effect of overfeeding on the protein metabolism of man. I. The effect of superimposing raw and boiled milks on a diet adequate for maintenance. *Biochem. J.*, 31: 681, 1937.
59. Cuthbertson, David Paton, Alexander McCutcheon, and Hamish Nisbet Munro. A study of the effect of overfeeding on the protein metabolism of man. II. The superimposition on a diet adequate for maintenance, of beef (or soya flour) +lactose +butter, equivalent in protein, carbohydrate and fat content to a litre of milk. *Biochem. J.*, 31: 689, 1937.
60. Cuthbertson, David Paton, and Hamish Nisbet Munro. A study of the effect of overfeeding on the protein metabolism of man. III. The protein-

saving effect of carbohydrate and fat when superimposed on a diet adequate for maintenance. *Biochem. J.*, 31: 694, 1937.

61. Daniels, Amy L., Mary K. Hutton, Elizabeth M. Knott, Olive E. Wright, Gladys J. Everson, and Florence Scoular. A study of the protein needs of preschool children. *J. Nutrition*, 9: 91, 1935.
62. Daniels, Amy L., Mary K. Hutton, Elizabeth M. Knott, Olive E. Wright, and Mary Forman. Calcium and phosphorus needs of preschool children. *J. Nutrition*, 10: 373, 1935.
63. Davidson, Jehiel, and J. A. LeClerc. The variation in the mineral content of vegetables. *J. Nutrition*, 11: 55, 1936.
64. Davis, Norma J. Calcium, phosphorus and nitrogen retention of children. Effects of acid-forming and base-forming diets. *Am. J. Dis. Child.*, 49: 611, 1935.
65. Dearborn, Walter F., and John W. M. Rothney. Predicting the Child's Development. Sci-Art Publishers, Cambridge, Mass., 1941.
66. Dill, D. B., J. W. Wilson, F. G. Hall, and Sid Robinson. Properties of the blood of negroes and whites in relation to climate and season. *J. Biol. Chem.*, 136: 449, 1940.
67. Donelson, Eva, Betty Nims, Helen A. Hunscher, Carroll F. Shukers, and Icie G. Macy. Simple methods for metabolic balance studies and their interpretation. *J. Home Econ.*, 23: 267, 1931.
68. DuBois, Eugene F. Basal Metabolism in Health and Disease. Third edition. Lea and Febiger, Philadelphia, 1936.
69. DuBois, Eugene F. Lane Medical Lectures: The Mechanism of Heat Loss and Temperature Regulation. Stanford University Press, Stanford, California, 1937.
70. Dunbar, H. Flanders. Emotions and Bodily Changes. A Survey of Literature on Psychosomatic Interrelationships, 1910–1933. Second edition, Columbia University Press, New York, 1938.
71. Eagle, Harry. Studies on blood coagulation. I. The rôle of prothrombin and of platelets in the formation of thrombin. *J. Gen. Physiol.*, 18: 531, 1934.
72. East, Bion R., and Hilda Kaiser. Relation of dental caries in rural children to sex, age and environment. *Am. J. Dis. Child.*, 60: 1289, 1940.
73. Eddy, Walter H., and Gilbert Dalldorf. The Avitaminoses—The Chemical, Clinical and Pathological Aspects of the Vitamin Deficiency Diseases. Second edition. The Williams and Wilkins Co., Baltimore, 1941.
74. Eisenbrand, J., and H. Picher. Polarographic estimation of biologically important ketones of the sterol group. *Z. Physiol. Chem.*, 260: 83, 1939.
75. Elman, Robert, and D. O. Weiner. Intravenous alimentation with special reference to protein (amino acid) metabolism. *J.A.M.A.*, 112: 796, 1939.
76. Elvehjem, C. A., and E. B. Hart. Synthetic rations and hemoglobin building. A note on the Drabkin-Waggoner modification of the Biazzo method for determining copper. *J. Biol. Chem.*, 91: 37, 1931.
77. Elvehjem, C. A., E. B. Hart, and W. C. Sherman. The availability of iron from different sources for hemoglobin formation. *J. Biol. Chem.*, 103: 61, 1933.
78. Elvehjem, C. A., E. B. Hart, and W. C. Sherman. The limitations of cereal-milk diets for hemoglobin formation. *J. Pediatrics*, 4: 65, 1934.
79. Eppright, Ercel S., and Arthur H. Smith. Influence of the inorganic salts in the diet on the composition of the ash of certain tissues of the rat. *J. Biol. Chem.*, 118: 679, 1937.
80. Erickson, Betty Nims, Harold H. Williams, Frances Cope Hummel, and Icie G. Macy. The lipid and mineral distribution in the serum and erythrocytes of normal children. *J. Biol. Chem.*, 118: 15, 1937.
81. Erickson, Betty Nims, Harold H. Williams, Frances Cope Hummel, Pearl Lee, and Icie G. Macy. The lipid and mineral distribution of the serum and erythrocytes in the hemolytic and hypochromic anemias of childhood. *J. Biol. Chem.*, 118: 569, 1937.
82. Erickson, Betty Nims, Harold H. Williams, Samuel S. Bernstein, Ira Avrin, Robert L. Jones, and Icie G. Macy. The lipid distribution of posthemolytic residue or stroma of erythrocytes. *J. Biol. Chem.*, 122: 515, 1937.

83. Erickson, Betty N., Harold H. Williams, Ira Avrin, and Pearl Lee. The lipid distribution of human platelets in health and disease. *J. Clin. Invest.*, 18: 81, 1939.

84. Erickson, Betty Nims, Ira Avrin, D. Maxwell Teague, and Harold H. Williams. Micromethods for the determination of sphingomyelin and choline. Applications for the estimation of the phospholipid partition (sphingomyelin, lecithin, and cephalin) in blood and tissues. *J. Biol. Chem.*, 135: 671, 1940.

85. Erickson, Betty Nims, Olive Hoffman, Eliot F. Beach, Harold H. Williams, and Icie G. Macy. Correlated hematologic, physical, and chemical studies of the blood and stroma in polycythemia and beef embryo. *J. Lab. Clin. Med.*, 26: 1492, 1940.

86. Erickson, Betty Nims, Helen J. Souders, Marion L. Shepherd, D. Maxwell Teague, and Harold H. Williams. Relation between cerebroside and neutral fat contents of blood plasma, erythrocytes and stroma. *Proc. Soc. Exper. Biol. Med.*, 45: 153, 1940.

87. Evans, Herbert M., and Samuel Lepkovsky. Sparing action of fat on the antineuritic vitamin B. *J. Biol. Chem.*, 83: 269, 1929.

88. Evans, Herbert M., and Samuel Lepkovsky. The sparing action of fat on vitamin B. II. The role played by the melting point and the degree of unsaturation of various fats. *J. Biol. Chem.*, 96: 165, 1932.

89. Evans, Herbert M., and Samuel Lepkovsky. The sparing action of fat on vitamin B. VIII. On the loss of vitamin B from the rat's tissues. *J. Biol. Chem.*, 108: 439, 1935.

90. Everson, Gladys J., and Amy L. Daniels. A study of manganese retentions in children. *J. Nutrition*, 8: 497, 1934.

91. Fairbanks, B. W., and H. H. Mitchell. The relation between calcium retention and the store of calcium in the body, with particular reference to the determination of calcium requirements. *J. Nutrition*, 11: 551, 1936.

92. Fantus, Bernard, Geza Kopstein, and Hilmar Schmidt. Roentgen study of intestinal motility as influenced by bran. *J.A.M.A.*, 114: 404, 1940.

93. Farr, Lee E. The intravenous administration of small doses of casein hydrolysate to nephrotic children and its effect upon the nitrogen balance and plasma amino acid level. *J. Pediatrics*, 16: 679, 1940.

94. Ferguson, John H. Observations on the alterations of blood platelets as a factor in coagulation of the blood. *Am. J. Physiol.*, 108: 670, 1934.

95. Ferguson, John H. An experimental analysis of coagulant activation. *Am. J. Physiol.*, 117: 587, 1936.

96. Ferris, Edmond J. Increase in lymphocytes in healthy persons under emotional states. *Am. J. Anatomy*, 63: 297, 1938.

97. Fischer, Hans and Adolf Stern. Die Chemie des Pyrrols. II. Band, Pyrrolfarbstoffe, 2. Hälfte. Akademische Verlagsgesellschaft, m.b.H., Leipzig, 1940.

98. Fisher, R. A., and F. Yates. Statistical Tables for Biological, Agricultural and Medical Research. Oliver and Boyd, London, 1938.

99. Fletcher, E. Gordon and A. Graeme Mitchell. Physiologic variations in the leukocytes in infants and in children. *Am. J. Dis. Child.*, 34: 807, 1927.

100. Flory, Charles D. Osseous Development in the Hand as an Index of Skeletal Development. Soc. Res. Child. Dev. Monographs, Vol. 1, No. 3, 1936.

101. Flosdorf, Earl W., and Stuart Mudd. An improved procedure and apparatus for preservation of sera, micro-örganisms and other substances. The Cryochem-Process. *J. Immunology*, 34: 469, 1938.

102. Folch, Jordi, and Donald D. Van Slyke. Preparation of blood lipid extracts free from non-lipid extractives. *Proc. Soc. Exper. Biol. Med.*, 41: 514, 1939.

103. Folch, Jordi, H. A. Schneider, and Donald D. Van Slyke. Notes on gasometric plasma lipid analyses. *J. Biol. Chem.*, 133: xxxiii, 1940.

104. Folin, Otto. Laws governing the chemical composition of urine. *Am. J. Physiol.*, 13: 66, 1905.

105. Folin, Otto. On sulphate and sulphur determinations. *J. Biol. Chem.*, 1: 131, 1905.

106. Folin, Otto. On the determination of creatinine and creatine in urine. *J. Biol. Chem.*, 17: 469, 1914.

107. Freyberg, R. H., and R. L. Grant. Loss of minerals through the skin of normal humans when sweating is avoided. *J. Clin. Invest.*, 16: 729, 1937.

108. Fries, Joseph H., and Zizmor, Judah. Roentgen studies of children with alimentary disturbances due to food allergy. *Am. J. Dis. Child.*, 54: 1239, 1937.

109. Gaebler, Oliver Henry, and W. H. Price. Effects of an anterior pituitary growth preparation on protein metabolism. *J. Biol. Chem.*, 121: 497, 1937.

110. Garelli, F. Le Dosage Du Soufre, Du Chlore Et Du Brome Dans Les Corps Organiques Au Moyen De La Bombe Calorimétrique. *Chimie et Industrie*, 27: 166S, 1932. Numéro Spécial Congress De Chimie Industrielle 11th, 1931.

111. Gerard, P. Teneur en potassium et en sodium des différents organes d'un chien. *Compt. rend. Acad.*, 154: 839, 1912.

112. Gershon-Cohen, J. and Harry Shay. Experimental studies on gastric physiology in man. III. A study of pyloric control. The role of milk and cream in the normal and in subjects with quiescent duodenal ulcer. *Am. J. Roentgen. Rad. Therapy*, 38: 427, 1937.

113. Gershon-Cohen, J., and Harry Shay. The effect of osmotic changes in the small intestine upon gastric emptying in man. *Am. J. Dig. Dis. Nutr.*, 4: 637, 1937.

114. Gilman, Henry. Some biological applications of organo-metallic compounds. *Science*, 93: 47, 1941.

115. Givens, Maurice H., and Lafayette B. Mendel. Studies in calcium and magnesium metabolism. I. The effects of base and acid. *J. Biol. Chem.*, 31: 421, 1917.

116. Guest, George M., Estelle W. Brown, and Mary Wing. Erythrocytes and hemoglobin of the blood in infancy and in childhood. II. Variability in number, size and hemoglobin content of the erythrocytes during the first five years of life. *Am. J. Dis. Child.*, 56: 529, 1938.

117. Gutman, Alexander B., and Margaret Low. On the approximation of the calculated to determined calcium content of human dietaries. *J. Nutrition*, 18: 257, 1939.

118. Haden, Russell L. The volume thickness index of the erythrocyte of man. *J. Lab. Clin. Med.*, 20: 567, 1934.

119. Hamil, B. M., M. W. Poole, Betty Munday, Marion L. Shepherd, Louise Emerson, Icie G. Macy, and T. E. Raiford. A consideration of quantitative relations between erythrocytes, leucocytes and hemoglobin of the blood. *J. Lab. Clin. Med.*, 23: 488, 1937.

120. Hamilton, Bengt, and Margaret Moriarty. The composition of growth in infancy. I. A premature infant. *Am. J. Dis. Child.*, 37: 1169, 1929.

121. Hamilton, Joseph G. The rates of absorption of the radioactive isotopes of sodium, potassium, chlorine, bromine and iodine in normal human subjects. *Am. J. Physiol.*, 124: 667, 1938.

122. Hanszen, Arthur. The bactericidal power of the stomach and some factors which influence it. *Am. J. Dig. Dis. Nutr.*, 1: 725, 1934.

123. Harris, Robert S., and John W. M. Bunker. Roentgenographic study of gastrointestinal motility in rachitic rats. *Am. J. Roentgen. Rad. Therapy*, 33: 25, 1935.

124. Hart, E. B., H. Steenbock, C. A. Elvehjem, and J. Waddell. Iron in nutrition. I. Nutritional anemia on whole milk diets and the utilization of inorganic iron in hemoglobin building. *J. Biol. Chem.*, 65: 67, 1925.

125. Hart, E. B., H. Steenbock, J. Waddell, and C. A. Elvehjem. Iron in nutrition. VII. Copper as a supplement to iron for hemoglobin building in the rat. *J. Biol. Chem.*, 77: 797, 1928.

126. Hastings, A. Baird, and Charles W. Eisele. Diurnal variations in the acid-base balance. *Proc. Soc. Exper. Biol. Med.*, 43: 308, 1940.

127. Haurowitz, F., and J. Sládek. Über die Chemische Zusammensetzung der Blutplattchen. *Z. Physiol. Chem.*, 173: 233, 1928.

128. Hawk, Philip B., and Olaf Bergeim. Practical Physiological Chemistry. A Book Designed for Use in Courses in Practical Physiological Chemistry

in Schools of Medicine and of Science. Tenth edition. P. Blakiston's Son and Co., Inc., Philadelphia, 1931.

129. Hawks, Jean E., Marie Dye, and Merle M. Bray. An improved technic for metabolism studies in pre-school children with a statistical determination of its reliability. *J. Nutrition*, 13: 51, 1937.

130. Heller, V. G., and Robert Wall. The indigestible carbohydrates of feeds. *J. Nutrition*, 19: 141, 1940.

131. Henderson, Lawrence J., and Walter W. Palmer. On the several factors of acid excretion. *J. Biol. Chem.*, 17: 305, 1914.

132. Henderson, Lawrence J. Blood, A Study in General Physiology. Yale University Press, New Haven, 1928.

133. Hess, Alfred F. The ultraviolet rays of the sun. *J.A.M.A.*, 84: 1033, 1925.

134. Heyrovský, J., and M. Shikata. Researches with the dropping mercury cathode. Part II. The polarograph. *Rec. Trav. chim. Pays-Bas*, 44: 496, 1925.

135. Heyrovský, J. A Polarographic Study of the Electrokinetic Phenomena of Adsorption, Electroreduction and Overpotential Displayed at the Dropping Mercury Cathode. Actualities Scientifiques et Industrielles, No. 90, Hermann et Cie., Paris, 1934.

136. Heyrovský, J., and D. Ilkovič. Polarographic studies with the dropping mercury electrode. Part II. The absolute determination of reduction and depolarization potentials. *Coll. Czech. Chem. Commun.*, 7: 198, 1935.

137. Heyrovský, J. Polarography. *In*, Boettger, W. Physical Methods of Chemical Analysis. Vol. II, Leipzig, 1936.

138. Hill, Elsie, and W. R. Bloor. Fat excretion. *J. Biol. Chem.*, 53: 171, 1922.

139. Hinrichs, M. A. Some effects of physical fatigue on peripheral circulation in athletes. *Proc. Soc. Exper. Biol. Med.*, 45: 685, 1940.

140. Hoagland, Charles L. Microdetermination of sulfate and phosphate by manometric combustion of their organic precipitates. *J. Biol. Chem.*, 136: 543, 1940.

141. Hobson, W. Urinary output of creatine and creatinine associated with physical exercise, and its relationship to carbohydrate metabolism. *Biochem. J.*, 33: 1425, 1939.

142. Hohn, Hans. Chemical Analysis with the Polarograph. J. Springer, Berlin, 1937.

143. Holt, L. Emmett, Jr. Growth and Development of the Child. Part III, Nutrition. White House Conference on Child Health and Protection. The Century Co., New York, 1932.

144. Holt, L. Emmett, Jr., Herbert C. Tidwell, Claude M. Kirk, Dorothea M. Cross, and Sarah Neale. Studies in fat metabolism. I. Fat absorption in normal infants. *J. Pediatrics*, 6: 427, 1935.

145. Holt, L. Emmett, Jr., Anthony A. Albanese, Joseph E. Brumback, Jr., Charlotte Kajdi, and Dorothy M. Wangerin. Nitrogen balance in experimental tryptophane deficiency in man. *Proc. Soc. Exper. Biol. Med.*, 48: 726, 1941.

146. Hopkins, Sir Frederick Gowland. Nutrition and human welfare. *Nutr. Abs. Rev.*, 1: 3, 1931.

147. Hopkins, Sir Frederick Gowland. Some chemical aspects of life. *Science*, 78: 219, 1933.

148. Horvath, S. M., and D. B. Dill. The conversion of creatinine into creatine in normal urine. *J. Lab. Clin. Med.*, 26: 1673, 1940.

149. Horvath, S. M., and W. Corwin. Creatinine-creatine excretion in schizophrenics. *Am. J. Physiol.*, 133: 679, 1941.

150. Houchin, O. B., and C. W. Turner. Effect of season (temperature) on blood lipids. *Proc. Soc. Exper. Biol. Med.*, 42: 477, 1939.

151. Hove, E., C. A. Elvehjem, and E. B. Hart. The relation of zinc to carbonic anhydrase. *J. Biol. Chem.*, 136: 425, 1940.

152. Howell, William H. A Text-Book of Physiology for Medical Students and Physicians. Ninth edition. W. B. Saunders Company, Philadelphia, 1924.

153. Howell, W. H. Theories of blood coagulation. *Physiol. Rev.*, 15: 435, 1935.

154. Hubbard, Roger S. The alkaline tide as a method of studying gastric acidity. *Arch. Int. Med.*, 46: 994, 1930.

155. Hummel, Frances Cope, Helen R. Sternberger, Helen A. Hunscher, and Icie G. Macy. Metabolism of women during the reproductive cycle. VII. Utilization of inorganic elements (A continuous case study of a multipara). *J. Nutrition*, 11: 235, 1936.

156. Hummel, Frances Cope, Helen A. Hunscher, Mary F. Bates, Priscilla Bonner, Icie G. Macy, and Joseph A. Johnston. A consideration of the nutritive state in the metabolism of women during pregnancy. *J. Nutrition*, 13: 263, 1937.

157. Hummel, Frances Cope, and H. H. Willard. Determination of iron in biological materials. The use of *o*-phenanthroline. *Ind. Eng. Chem.*, Analy. Ed., 10: 13, 1938.

158. Hummel, Frances C., Marion L. Shepherd, and Icie G. Macy. Effect of changes in food intakes upon the lignin, cellulose, and hemicellulose contents of diets. *J. Am. Dietet. Assoc.*, 16: 199, 1940.

159. Hunscher, Helen A., Frances Cope Hummel, and Icie G. Macy. Variability of metabolic response of different children to a given intake of calcium. *Proc. Soc. Exper. Biol. Med.*, 35: 189, 1936.

160. Hunscher, Helen A., Frances Cope Hummel, and Icie G. Macy. Effect of varied banana intake on nitrogen and mineral balances of normal children. *Am. J. Dis. Child.*, 60: 509, 1940.

161. Hunter, Andrew. Creatine and Creatinine. Monographs on Biochemistry. Longmans, Green and Co., Ltd., London, 1928.

162. Hutchison, H. S. Fat metabolism in health and disease with special reference to infancy and childhood. *Quart. J. Med.*, 13: 277, 1919.

163. Ilkovič, D., and G. Semerano. Polarographic studies with the dropping mercury cathode. Part XXV. Increased sensitivity of microanalytical estimations by a compensation current. *Coll. Czech. Chem. Commun.*, 4: 176, 1932.

164. Ilkovič, D. Polarographic studies with the dropping mercury electrode. Part XLIV. The dependence of limiting currents on the diffusion constant, on the rate of dropping and on the size of drops. *Coll. Czech. Chem. Commun.*, 6: 498, 1934.

165. Irving, Laurence. The relation of solubility to the absorption of calcium salts from the intestine. *J. Biol. Chem.*, 68: 513, 1926.

166. Jeans, P. C., and Genevieve Stearns. The human requirement of vitamin D. *J.A.M.A.*, 111: 703, 1938.

167. Johnston, C. G., and I. S. Ravdin. Action of glucose on emptying of stomach. Effect of varying concentrations in both normal stomachs and after various gastric operations. *Ann. Surgery*, 101: 500, 1935.

168. Jorpes, Erik. The protein component of the erythrocyte membrane or stroma. *Biochem. J.*, 26: 1488, 1932.

169. Jukes, Thomas H. The distribution of pantothenic acid in certain products of natural origin. *J. Nutrition*, 21: 193, 1941.

170. Kaltreider, Nolan L. and George R. Meneely. The effect of exercise on the volume of the blood. *J. Clin. Inv.*, 19: 627, 1940.

171. Kapp, Eleanor M. Spectrographic observations on urinary porphyrins: the nature of a combined form of coproporphyrin found in urine. *Brit. J. Exper. Path.*, 20: 33, 1939.

172. Kato, Katsuji. Leucocytes in infancy and childhood. A statistical analysis of 1,081 total and differential counts from birth to fifteen years. *J. Pediatrics*, 7: 7, 1935.

173. Keilin, D., and T. Mann. Carbonic anhydrase. *Nature*, 144: 442, 1939.

174. Keilin, D., and T. Mann. Carbonic anhydrase. Purification and nature of the enzyme. *Biochem. J.*, 34: 1163, 1940.

175. Kelly, Harriet J. Anatomic age and its relation to stature. *Univ. Iowa Studies*, Vol. XII, No. 5, 1937.

176. Kenyon, Fanny, Charles A. Wilson, and Icie G. Macy. Daily fluctuations in urinary *pH*. *Arch. Pediatrics*, 51: 490, 1934.

177. Kenyon, Fanny, and Icie G. Macy. Hourly physiologic variations in

peripheral hemoglobin, red and white cell counts of women: Effect of pregnancy upon blood counts. *Human Biology*, 10: 511, 1938.

178. King, Helen. A new method of recording children's footprints. *Am. J. Dis. Child.*, 43: 89, 1932.

179. Kinsman, Gladys, Dorothy Sheldon, Elizabeth Jensen, Marie Bernds, Julia Outhouse, and H. H. Mitchell. The utilization of the calcium of milk by pre-school children. *J. Nutrition*, 17: 429, 1939.

180. Kirk, Esben. Gasometric microdetermination of phosphoric acid *J. Biol. Chem.*, 106: 191, 1934.

181. Kirk, Esben, Irvine H. Page, and Donald D. Van Slyke. Gasometric microdetermination of lipids in plasma, blood cells, and tissues. *J. Biol. Chem.*, 106: 203, 1934.

182. Kirk, Esben. A study on Kimmelstiel's procedure for titrimetric cerebroside determination, with description of an improved technique. *J. Biol. Chem.*, 123: 613, 1938.

183. Kodíček, E., and K. Wenig. Polarographic estimation of vitamin C. *Nature*, 142: 35, 1938.

184. Kohler, G. O., C. A. Elvehjem, and E. B. Hart. Modifications of the bipyridine method for available iron. *J. Biol. Chem.*, 113: 49, 1936.

185. Kolthoff, I. M., and James J. Lingane. The fundamental principles and applications of electrolysis with the dropping mercury electrode and Heyrovský's polarographic method of chemical analysis. *Chem. Rev.*, 24: 1, 1939.

186. Kolthoff, I. M., and James J. Lingane. Polarography. Interscience Publishers, Inc., New York, 1941.

187. Kon, Stanislaus K. On the carbon:nitrogen (C/N) ratio in the urine of rats deprived of one or both factors of the vitamin B complex. *J. Nutrition*, 1: 467, 1928.

188. Krakower, A. Fecal "fat" and its relation to fat in the diet. *Am. J. Physiol.*, 107: 49, 1934.

189. Kramer, Benjamin, and Frederick F. Tisdall. A clinical method for the quantitative determination of potassium in small amounts of serum. *J. Biol. Chem.*, 46: 339, 1921.

190. Kücera, Gottlieb. Zur oberflächenspannung von polarisiertem quecksilber. *Ann. Physik*, 11: 529, 1903.

191. Ladd, Maynard. Influence of the variations of diet on gastric motility in infants. *Tr. Am. Pediat. Soc.*, 25: 74, 1913.

192. Leathes, J. B. Croonian lectures on the rôle of fats in vital phenomena. *The Lancet*, 208,I: 803, 853, 957, 1019; 1925.

193. Lee, Milton O., and Norwood K. Schaffer. Anterior pituitary growth hormone and the composition of growth. *J. Nutrition*, 7: 337, 1934.

194. Lee, Pearl, and Betty Nims Erickson. Platelet studies in normal men and women (menstruating and non-menstruating) and subjects with bleeding disorders. Counts, disintegration rates, and intradermal platelet injections. *J. Lab. Clin. Med.*, 24: 821, 1938.

195. Levine, S. Z., M. A. Wheatley, T. H. McEachern, H. H. Gordon, and E. Marples. Respiratory metabolism in infancy and in childhood. XXI. Daily water exchange of normal infants. *Am. J. Dis. Child.*, 56: 83, 1938.

196. Levine, S. Z., Harry H. Gordon, and Eleanor Marples. A defect in the metabolism of tyrosine and phenylalanine in premature infants. II. Spontaneous occurrence and eradication by vitamin C. *J. Clin. Invest.*, 20: 209, 1941.

197. Lewis, Robert C., Gladys M. Kinsman, and Alberta Iliff. The basal metabolism of normal boys and girls from two to twelve years old, inclusive. *Am. J. Dis. Child.*, 53: 348, 1937.

198. Lewis, Samuel J. Thumb-sucking: A cause of malocclusion in the deciduous teeth. *J. Am. Dent. Assoc.*, 17: 1060, 1930.

199. Lewis, Samuel J. Undesirable habits influencing the deciduous dentition. *J. Am. Dent. Assoc.*, 18: 1766, 1931.

200. Lewis, Samuel J., and Ira A. Lehman. A quantitative study of the relation between certain factors in the development of the dental arch and the

occlusion of the teeth. *Internat. J. Orthodont., Oral Surg., Radiography*, 18: 1015, 1932.

201. Lewis, Samuel J. Some aspects of dental arch growth. *J. Am. Dent. Assoc.*, 23: 277, 1936.
202. Lindquist, E. F. Statistical Analysis in Educational Research. Houghton Mifflin Co., New York, 1940.
203. Lingane, James J., and I. M. Kolthoff. Fundamental studies with the dropping mercury electrode. II. Migration current. *J. Am. Chem. Soc.*, 61: 1045, 1939.
204. Lingane, James J., and Herbert Kerlinger. Use of a condenser to reduce galvanometer oscillations in polarographic measurements. *Ind. Eng Chem.*, Analyt. Ed., 12: 750, 1940.
205. Lingane, James J., and Orris L. Davis. Polarographic determination of Riboflavin (vitamin B) and other vitamin B factors. *J. Biol. Chem.*, 137: 567, 1941.
206. Lusk, Graham. The Elements of the Science of Nutrition. Fourth edition. W. B. Saunders Co., Philadelphia, 1928.
207. Maas, J. Polarographic studies with the dropping mercury cathode. LXXI. An empirical study of the factors of the diffusion current. *Coll. Czech. Chem. Commun.*, 10: 42, 1938.
208. McCance, R. A., and R. D. Lawrence. The carbohydrate content of foods. *Med. Res. Council, Spec. Rep. Ser.*, No. 135, London, 1929.
209. McCance, R. A. Experimental sodium chloride deficiency in man. *Proc. Royal Soc. London*, Series B., 119: 245, 1936
210. McCance, R. A., E. M. Widdowson, and L. R. B. Shackleton. The nutritive value of fruits, vegetables and nuts. *Med. Res. Council, Spec. Rep. Ser.*, No. 213, London, 1936.
211. McCance, R. A., and E. M. Widdowson. Absorption and excretion of iron. *The Lancet*, 233, II: 680, 1937.
212. McCance, R. A., and E. M. Widdowson. The absorption and excretion of iron following oral and intravenous administration. *J. Physiol.*, 94: 148, 1938.
213. McCollum, E V., Elsa Orent-Keiles, and Harry G. Day. The Newer Knowledge of Nutrition. Fifth edition. The Macmillan Company, New York, 1939.
214. McDougall, Edith Jean. The counteraction by fat of the anticalcifying action of cereals. *Biochem. J.*, 32: 194, 1938.
215. McGowan, John Pool. Some considerations regarding and investigations into calcium and phosphorus metabolism. *Biochem. J.*, 27: 934, 1933.
216. McHenry, E. W. Vitamin B_1 and the synthesis of fat from carbohydrate. *Science*, 86: 200, 1937.
217. MacKay, E. M., and A. M. Butler. A comparison of the mineral (base) composition of dietaries as determined from tables and by analysis. *J. Am. Dietet. Assoc.*, 10: 398, 1935.
218. Macy, Icie G., Helen A. Hunscher, Betty Nims, and Sylvia Schimmel McCosh. Metabolism of women during the reproductive cycle. I. Calcium and phosphorus utilization in pregnancy. *J. Biol. Chem.*, 86: 17, 1930.
219. Macy, Icie G., Eva Donelson, M. Louisa Long, Alice Graham, Mary E. Sweeny, and M. Margaret Shaw. Nitrogen, calcium and phosphorus balances in late gestation under a specified dietary régime. A record of one case. *J. Am. Dietet. Assoc.*, 6: 314, 1931.
220. Macy, Icie G., Helen A. Hunscher, Frances Cope Hummel, Mary F. Bates, and Marsh W. Poole. Is the metabolic nitrogen balance an accurate measure for adequate protein consumption in children? *J. Nutrition*, 11: Supp., p. 6, 1936.
221. Macy, Icie G., Lawrence Reynolds, and Helen J. Souders. The effect of carmine upon the gastrointestinal motility of children. *Am. J. Physiol.*, 126: 75, 1939.
222. Macy, Icie G., Frances Cope Hummel, Helen A. Hunscher, Marion L. Shepherd, and Helen J. Souders. Effects of simple dietary alterations

upon retention of positive and negative minerals by children. *J. Nutrition*, 19: 461, 1940.

223. Macy, Icie G., Lawrence Reynolds, Helen J. Souders, and Mary Bates Olson. Normal variation in the gastrointestinal response of healthy children. *Am. J. Roentgen. Rad. Therapy*, 43: 394, 1940.

224. Maizels, Montague. The anion and cation contents of normal and anaemic bloods. *Biochem. J.*, 30: 821, 1936.

225. Majer, V. Micropolarographic studies. I. Apparatus and technic. *Mikrochemie*, 18: 74, 1935.

226. Majer, V. Polarographic studies with the dropping mercury cathode. Part XLVI. Current-voltage curves obtained with small anodes. *Coll. Czech. Chem. Commun.*, 7: 146, 1935.

227. Majer, V. Polarographic studies with the dropping mercury cathode. Part XLVII. Passivation of small mercury anodes. *Coll. Czech. Chem. Commun.*, 7: 215, 1935.

228. Majer, V. Polarographic studies with the dropping mercury cathode. Part LXXI. Changes of polarization when small anodes are used. *Coll. Czech. Chem. Commun.*, 9: 360, 1937.

229. Mangold, Ernst. The digestion and utilization of crude fibre. *Nutr. Abst. Rev.*, 3: 647, 1934.

230. Mann, Frank C., and Jesse L. Bollman. A symposium concerned with the duodenal factors in neutralization of acid chyme. *Am. J. Dig. Dis. Nutr.*, 2: 284, 1935.

231. Manville, Ira A., Elizabeth M. Bradway, and Avoca S. McMinis. Pectin as a detoxication mechanism. *Am. J. Dig. Dis. Nutr.*, 3: 570, 1936.

232. Manville, Ira A., and Walter R. Munroe. Studies on the gastric hunger mechanism. II. The inhibitory effect of dextrose solutions. *Am. J. Dig. Dis. Nutr.*, 4: 561, 1937.

233. Massler, M., I. Schour, and H. G. Poncher. Developmental pattern of the child as reflected in the calcification pattern of the teeth. *Am. J. Dis. Child.*, 62: 33, 1941.

234. Mathews, Albert P. Physiological Chemistry. A Text-Book and Manual for Students. Fifth edition. William Wood and Company, New York, 1930.

235. Mendel, Lafayette B. Some aspects of the newer physiology of the gastrointestinal canal. *J.A.M.A.*, 43: 1539, 1904.

236. von Meysenbug, L., and Archie Fine. Banana powder and the fecal flora of infants. *J. Pediatrics*, 8: 630, 1936.

237. Mills, C. A. Environmental temperatures and thiamine requirements. *Am. J. Physiol.*, 133: 525, 1941.

238. Mitchell, H. H., and T. S. Hamilton. The Biochemistry of the Amino Acids. The Chemical Catalog Co., Inc., New York, 1929.

239. Mora, Jacob M., Leo E. Amtman, and Samuel F. Hoffman. Effect of mental and emotional states on the leukocyte count. I. Preliminary report. *J.A.M.A.*, 86: 945, 1926.

240. Morey, Nancy Booker. An analysis and comparison of different methods of calculating the energy value of diets. *Nutr. Abst. Rev.*, 6: 1, 1936.

241. Morgan, Agnes Fay. The water-soluble vitamins. *Ann. Rev. Biochem.*, 10: 337, 1941.

242. Morrison, Dempsie B., and Thomas P. Nash, Jr. The copper content of infant livers. *J. Biol. Chem.*, 88: 479, 1930.

243. Mueller, E. F. Dropping electrode with a constant head of mercury. *Ind. Eng. Chem.*, Analyt. Ed., 12: 171, 1940.

244. Mugrage, Edward R., and Marjory I. Andresen. Values for red blood cells of average infants and children. *Am. J. Dis. Child.*, 51: 775, 1936.

245. Munday, Betty, Marion L. Shepherd, Louise Emerson, Brenton M. Hamil, Marsh W. Poole, Icie G. Macy, and T. E. Raiford. Hemoglobin differences in healthy white and negro infants. *Am. J. Dis. Child.*, 55: 776, 1938.

246. Munsell, Hazel E. Vitamins and their occurrence in foods. *Milbank Memorial Fund Quarterly*, 18: 311, 1940.

247. Murlin, John R. Physiology of metabolism in infancy and childhood. *In*, Abt's Pediatrics, Vol. I. W. B. Saunders Co., Philadelphia, 1923.
248. Newburgh, L. H., and Frances MacKinnon. The Practice of Dietetics. The Macmillan Co., New York, 1934.
249. Newburgh, L. H., M. W. Johnston, F. H. Lashmet, and J. M. Sheldon. Further experiences with the measurement of heat production from insensible loss of weight. *J. Nutrition*, 13: 203, 1937.
250. Newburgh, L. H., and Margaret Woodwell Johnston. The insensible loss of water. *Physiol. Rev.*, 22: 1, 1942.
251. Nicholls, Lucius, and Ananda Nimalasuriya. Adaptation to a low calcium intake in reference to the calcium requirements of a tropical population. *J. Nutrition*, 18: 563, 1939.
252. Noll, C. I., and O. G. Jensen. The chemical determination of nicotinic acid in milk and milk derivatives. *J. Biol. Chem.*, 140: 755, 1941.
253. Offenkrantz, Frederick M., and Maxwell Karshan. Serum cholesterol values for children. *Am. J. Dis. Child.*, 52: 784, 1936.
254. Olmsted, William H., and Ray David Williams. Carbohydrates of certain vegetables and fruits. *Proc. Soc. Exper. Biol. Med.*, 40: 586, 1939.
255. Orr, W. J., L. E. Holt, Jr., L. Wilkins, and F. H. Boone. The relation of calcium and phosphorus in the diet to the absorption of these elements from the intestine. *Am. J. Dis. Child.*, 28: 574, 1924.
256. Osborne, Thomas B., and Lafayette B. Mendel. Amino-acids in nutrition and growth. *J. Biol. Chem.*, 17: 325, 1914.
257. Osgood, Edwin E. Normal hematologic standards. *Arch. Int. Med.*, 56: 849, 1935.
258. Osgood, Edwin E., and Russell L. Baker. Erythrocyte, hemoglobin, cell volume and color, volume and saturation index standards for normal children of school age. *Am. J. Dis. Child.*, 50: 343, 1935.
259. Page, Irvine H., Esben Kirk, William H. Lewis, Jr., William R. Thompson, and Donald D. Van Slyke. Plasma lipids of normal men at different ages. *J. Biol. Chem.*, 111: 613, 1935.
260. Palmer, Walter W., James H. Means, and James L. Gamble. Basal metabolism and creatinine elimination. *J. Biol. Chem.*, 19: 239, 1914.
260*a*. Parpart, Arthur K. The preparation of red cell membranes. *J. Cellular Comp. Physiol.*, 19: 248, 1942.
261. Pearson, Karl. Tables for Statisticians and Biometricians, Part II. Biometric Laboratory, University College, London. Cambridge University Press, 1931.
262. Peters, John P., and Donald D. Van Slyke. Quantitative Clinical Chemistry, Vol. I. Interpretations. The Williams and Wilkins Co., Baltimore, 1931.
263. Peters, John P., and Donald D. Van Slyke. Quantitative Clinical Chemistry. Vol. II. Methods. The Williams and Wilkins Co., Baltimore, 1932.
264. Peters, John P. Body Water. The Exchange of Fluids in Man. Charles C Thomas, Springfield, Ill., 1935.
265. Petersen, William F. The Patient and the Weather, Vol. I, Part 2, Autonomic Integration. Edwards Brothers, Inc., Ann Arbor, 1936.
266. Peterson, W. H., and C. A. Hoppert. The loss of mineral and other constituents from vegetables by various methods of cooking. *J. Home Econ.*, 17: 265, 1925.
267. Ponder, Eric. The Mammalian Red Cell and the Properties of Haemolytic Systems. Verlag von Gebrüder Borntraeger, Berlin, 1934.
268. Ponder, Eric. The kinetics of hemolysis. *Physiolog. Rev.*, 16: 19, 1936.
269. Poole, Marsh W., Betty N. Erickson, Harold H. Williams, H. G. Burkholder, and T. Leucutia. Ten years of treatment and progress in a case of chronic myeloid leukemia. The lipid distribution in leukocytes and erythrocytes. *J. Mich. State Med. Soc.*, 37: 993, 1938.
270. Porter-Levin, Thelma. Calcium and phosphorus metabolism of normal pre-school children: II. Successive balance studies showing the range of variation in calcium and phosphorus storage. *J. Am. Diet. Assoc.*, 9: 22, 1933.
271. Porter, Thelma. Iron balances on four normal pre-school children. *J. Nutrition*, 21: 101, 1941.

272. Quigley, J. P., H. J. Zettelman, and A. C. Ivy. Analysis of the factors involved in gastric motor inhibition by fats. *Am. J. Physiol.*, 108: 613, 1934.

273. Quigley, J. P., and K. R. Phelps. The mechanism of gastric motor inhibition from ingested carbohydrates. *Am. J. Physiol.*, 109: 133, 1934.

274. Ramage, H., J. H. Sheldon, and Wilfrid Sheldon. A spectrographic investigation of the metallic content of the liver in childhood. *Proc. Roy Soc. London*, Series *B*, 113: 308, 1933.

275. Ravdin, I. S., C. G. Johnston, and P. J. Morrison. Comparison of concentration of glucose in the stomach and intestine after intragastric administration. *Proc. Soc. Exper. Biol. Med.*, 30: 955, 1932.

276. Ray, Thomas W. The determination of manganese in organic material containing large amounts of calcium and chlorides. The determination of manganese in the mouse and in milk. *J. Biol. Chem.*, 134: 677, 1940.

277. Reynolds, Lawrence, Icie G. Macy, Helen Hunscher, and Mary Bates Olson. The gastrointestinal response of average, healthy children to test meals of barium in milk, cream, meat, and carbohydrate media *Am. J. Roentgen. and Rad. Therapy*, 43: 517, 1940.

278. Rietz, H. L., and H. H. Mitchell. On the metabolism experiment as a statistical problem. *J. Biol. Chem.*, 8: 297, 1910.

279. Robertson, Elizabeth Chant. Intestinal stasis due to low mineral intake. *Am. J. Dis. Child.*, 53: 500, 1937.

280. Rose, Mary S., Grace MacLeod, Ella McC. Vahlteich, Esther H. Funnell and Catherine L. Newton. The influence of bran on the alimentary tract. *J. Am. Diet. Assoc.*, 8: 133, 1932.

281. Rose, Mary Swartz. The Foundations of Nutrition. Revised Edition. The Macmillan Co., New York, 1933.

282. Rose, William C. The Significance of the Amino Acids in Nutrition. *The Harvey Lectures*, Series XXX: 49, 1934. The Williams and Wilkins Co., Baltimore, 1936.

283. Rose, William C. The nutritive significance of the amino acids. *Physiol. Rev.*, 18: 109, 1938.

284. Rose, Wm. C., and Eldon E. Rice. The significance of the amino acids in canine nutrition. *Science*, 90: 186, 1939.

285. Rosenthal, H. G. Polarographic determination of the disulfide and sulfhydryl groups in biological substances. *Mikrochemie*, 22: 233, 1937.

286. Rowe, Albert H. Roentgen studies of patients with gastro-intestinal food allergy. *J.A.M.A.*, 100: 394, 1933.

287. Rowe, Albert H. Clinical Allergy Due to Foods, Inhalants, Contactants, Fungi, Bacteria and Other Causes. Manifestations, Diagnosis and Treatment. Lea and Febiger, Philadelphia, 1937.

288. Scales, F. M., and A. P. Harrison. Boric acid modification of the Kjeldahl method for crop and soil analysis. *J. Ind. Eng. Chem.*, 12: 350, 1920.

289. Scammon, Richard E. A summary of the anatomy of the infant and child. *In*, Abt's. Pediat., Vol. I. W. B. Saunders Co., Philadelphia, 1923.

290. Schilling, Victor. The Blood Picture and Its Clinical Significance. *Translated and edited by* R. B. H. Gradwohl. C. V. Mosby Co., St. Louis, 1929.

291. Schlutz, Frederic W., Minerva Morse, and John L. Gedgoud. Some responses of the child to exercise. *J. Pediatrics*, 16: 729, 1940.

292. Schneider, Burch. The subdivision of the metabolic nitrogen in the feces of the rat, swine, and man. *J. Biol. Chem*, 109: 249, 1935.

293. Schneider, Howard A., J. Katherine Ascham, Blanche R. Platz, and H. Steenbock. The anti-acrodynic properties of certain foods. *J. Nutrition*, 18: 99, 1939.

294. Schoenheimer, Rudolf, and Warren M. Sperry. A micromethod for the determination of free and combined cholesterol. *J. Biol. Chem.*, 106: 745, 1934.

295. Schultze, M. O. The effect of deficiencies in copper and iron on the cytochrome oxidase of rat tissues. *J. Biol. Chem.*, 129: 729, 1939.

296. Schultze, M. O., and K. A. Kuiken. The effect of deficiencies in copper and iron on the catalase activity of rat tissues. *J. Biol. Chem.*, 137: 727, 1941.

297. Scott, Wilfred W. Standard Methods of Chemical Analysis. A Manual of Analytical Methods and General Reference for the Analytical Chemist and for the Advanced Student. Vol. 1, The Elements. Fourth edition. D. Van Nostrand Co., New York, 1927.
298. Scoular, Florence I. A quantitative study, by means of spectrographic analysis, of zinc in nutrition. *J. Nutrition,* 17: 103, 1939.
299. Sealock, Robert Ridgely, Jesse D. Perkinson, Jr., and Daniel H. Basinski. Further analysis of the rôle of ascorbic acid in phenylalanine and tyrosine metabolism. *J. Biol. Chem.,* 140: 153, 1941.
300. Semerano, G. The Polarograph, Its Theory and Applications. A. Draghli, Padua, 1933.
301. Shaffer, Philip. The Excretion of Kreatinin and Kreatin in Health and Disease. *Am. J. Physiol.,* 23: 1, 1908.
302. Shaffer, Philip A., and Michael Somogyi. Copper-iodometric reagents for sugar determination. *J. Biol. Chem.,* 100: 695, 1933.
303. Shapiro, Arthur, Harry Koster, D. Rittenberg, and Rudolf Schoenheimer. The origin of fecal fat in the absence of bile, studied with deuterium as an indicator. *Am. J. Physiol.,* 117: 525, 1936.
304. Sharp, E. A., and E. M. Schleicher. Bock's erythrocytometer and technic for its use. *J. Lab. Clin. Med.,* 21: 975, 1935.
305. Shepherd, Marion L., Frances Cope Hummel, and Icie G. Macy. Influence of raw banana and apple upon disappearance of complex carbohydrates from the alimentary tracts of normal children. *Am. J. Dig. Dis.,* 7: 248, 1940.
306. Sherman, H. C., and L. E. Booher. The calcium content of the body in relation to that of the food. *J. Biol. Chem.,* 93: 93, 1931.
307. Sherman, Henry C. Chemistry of Food and Nutrition. Fifth edition. The Macmillan Co., New York, 1937.
308. Sherman, Henry C., and Caroline Sherman Lanford. Essentials of Nutrition. The Macmillan Company, New York, 1940.
309. Sherman, Henry C. Chemistry of Food and Nutrition. Sixth edition. The Macmillan Co., New York, 1941.
310. Sherman, W. C., C. A. Elvehjem, and E. B. Hart. Further studies on the availability of iron in biological materials. *J. Biol. Chem.,* 107: 383, 1934.
311. Shields, J. B., B. W. Fairbanks, G. H. Berryman, and H. H. Mitchell. The utilization of calcium in carrots, lettuce and string beans in comparison with the calcium in milk. *J. Nutrition,* 20: 263, 1940.
312. Shohl, Alfred T. Mineral Metabolism. Reinhold Publishing Company, New York, 1939.
313. Shohl, Alfred T., Allan M. Butler, Kenneth D. Blackfan, and Elsie MacLachlan. Nitrogen metabolism during the oral and parenteral administration of the amino acids of hydrolyzed casein. *J. Pediatrics,* 15: 469, 1939.
314. Short, James J. Diurnal variations in concentration of red blood cells and hemoglobin. *J. Lab. Clin. Med.,* 20: 708, 1934.
315. Siebert, H., and I. Langer. The use of the new capillary tubes in polarography. *Chem. Fabrik.,* 11: 141, 1938.
316. Sinclair, R. G. The physiology of the phospholipids. *Physiol. Rev.,* 14: 351, 1934.
317. Smith, Arthur H. Phenomena of retarded growth. Editorial Review. *J. Nutrition,* 4: 427, 1931.
318. Smith, Margaret Cammack, and Louise Otis. Hemoglobin regeneration in anemic rats in relation to iron intake with suggestions for improvement of the bioassay technic for measuring available iron. *J. Nutrition,* 13: 573, 1937.
319. Smuts, D. B. The relation between the basal metabolism and the endogenous nitrogen metabolism, with particular reference to the estimation of the maintenance requirement of protein. *J. Nutrition,* 9: 403, 1935.
320. Snedecor, George W. Calculation and interpretation of analysis of variance and covariance. Collegiate Press, Inc., Ames, Iowa, 1934.

321. Snyder, Marshall L. A simple colorimetric method for the estimation of relative numbers of lactobacilli in the saliva. *J. Dent. Research*, 19: 349, 1940.

322. Snyder, Marshall L. A simple colorimetric method for the diagnosis of caries activity. *J. Am. Dent. Assoc.*, 28: 44, 1941.

323. Sorg-Matter, H. Loi quantitative de la dépense azoteé minima des homéothermes; Validite intraspecifique. *Arch. Internat. Physiol.*, T. 30: 126, 1928.

324. Sperry, Warren M. Cholesterol of the blood plasma in the neonatal period. *Am. J. Dis. Child.*, 51: 84, 1936.

325. Sperry, Warren M. The relationship between total and free cholesterol in human blood serum. *J. Biol. Chem.*, 114: 125, 1936.

325*a*. Sperry, Warren M. The determination of cholesterol. *J. Biol. Chem.*, 118: 377, 1937.

325*b*. Sperry, Warren M. A micromethod for the determination of total and free cholesterol. *Am. J. Clin. Path.*, Tech. Suppl., 8 (2): 91, 1938.

326. Stadie, William C., John A. Zapp, Jr., and Francis D. W. Lukens. Intermediary metabolism in diabetes mellitus. The non-formation of acetic acid and the ratio of ketone body increase to fatty acid decrease in livers of diabetic animals. *J. Biol. Chem.*, 137: 75, 1941.

327. Stearns, Genevieve. A rapid method for the preparation of fecal digests suitable for use in nitrogen and mineral analyses. *J. Lab. Clin. Med.*, 14: 954, 1929.

328. Stearns, Genevieve. The significance of the retention ratio of calcium: phosphorus in infants and in children. *Am. J. Dis. Child.*, 42: 749, 1931.

329. Stearns, Genevieve, and Dorothy L. R. Moore. Growth in height and weight, and retention of nitrogen, calcium and phosphorus during recovery from severe malnutrition. Report of a case. *Am. J. Dis. Child.*, 42: 774, 1931.

330. Stearns, Genevieve. The mineral metabolism of normal infants. *Physiol. Rev.*, 19: 415, 1939.

331. Steggerda, F. R., and H. H. Mitchell. The calcium requirement of adult man and the utilization of the calcium in milk and in calcium gluconate. *J. Nutrition*, 17: 253, 1939.

332. Stern, Adolph, Eliot F. Beach, and Icie G. Macy. Polarographic microdetermination of cystine in protein hydrolysates. *J. Biol. Chem.*, 130: 733, 1939.

333. Stern, Adolph, and Eliot F. Beach. Microdetermination of homocystine in pure solution with the dropping mercury cathode. *Proc. Soc. Exper. Biol. Med.*, 43: 104, 1940.

334. Stern, Adolph. Indirect polarographic determination of phosphorus in biological material. *Ind. Eng. Chem.*, Anal. Ed., 14: 74, 1942.

335. Swanson, William W. The composition of growth II. The full-term infant. *Am. J. Dis. Child.*, 43: 10, 1932.

336. Swanson, W. W., and L. V. Iob. Loss of minerals through the skin of infants *Am. J. Dis. Child.*, 45: 1036, 1933.

337. Sweeny, Mary E., Helen King, Charles A. Wilson, and Lucea Hejinian. A Method of Recording the Posture of Pre-school Children. The Merrill-Palmer School Publication, Detroit, 1929.

338. Talbot, Fritz B., Edwin B. Wilson, and Jane Worcester. Basal metabolism of girls. Physiologic background and application of standards. *Am. J. Dis. Child.*, 53: 273, 1937.

339. Talbot, Fritz B. Basal metabolism standards for children. *Am. J. Dis. Child.*, 55: 455, 1938.

340. Talbot, Nathan B. Basal energy metabolism and creatinine in the urine. I. Observations on children. *Am. J. Dis. Child.*, 52: 16, 1936.

341. Talbot, Nathan B., Jane Worcester, and Ann Stewart. New creatinine standard for basal metabolism and its clinical application. *Am. J. Dis. Child.*, 58: 506, 1939.

342. Telfer, S. V. Studies in calcium and phosphorus metabolism. Part IV. The influence of free fatty acids in the intestine on the absorption and excretion of the mineral elements. *Quart. J. Med.*, 20: 1, 1926.

343. Thanheiser, G., and G. Maassen. The use of the polarograph in the steel-plant laboratory. I. Determination of copper, nickel, and cobalt simultaneously in steels. *Mitt. Kaiser-Wilhelm Inst. Eisenforschung Dusseldorf*, 19: 27, 1937.

344. Thannhauser, S. J., Joseph Benotti, and Harold Reinstein. Studies on animal lipids. XIV. The determination of lecithin, cephalin and sphingomyelin in body fluids and tissues; with analyses of normal human sera. *J. Biol. Chem.*, 129: 709, 1939.

345. Tidwell, Herbert C., and L. Emmett Holt, Jr. The estimation of the total lipids and the lipid partition in feces. *J. Biol. Chem.*, 112: 605, 1935.

346. Tisdall, Frederick F., and Alan Brown. Relation of the altitude of the sun to its antirachitic effect. *J.A.M.A.*, 92: 860, 1929.

347. Tocantins, Leandro M. Platelets and the structure and physical properties of blood clots. *Am. J. Physiol.*, 114: 709, 1935.

348. Todd, T. Wingate. Behavior Patterns of the Alimentary Tract. The Williams and Wilkins Co., Baltimore, 1930.

349. Todd, T. Wingate. Anthropology and growth. *Science*, 81: 259, 1935.

350. Todd, T. Wingate. Atlas of Skeletal Maturation. The C. V. Mosby Co., St. Louis, 1937.

351. Toepfer, E. W., and H. C. Sherman. The effect of liberal intakes of calcium or calcium and phosphorus on growth and body calcium. *J. Biol. Chem.*, 115: 685, 1936.

352. Treichler, Ray, and H. H. Mitchell. The influence of plane of nutrition and of environmental temperature on the relationship between basal metabolism and endogenous nitrogen metabolism subsequently determined. *J. Nutrition*, 22: 333, 1941.

353. Ujsaghy, P. Der Eiweissstoffwechsel des Säuglings im Spiegel des Stickstoff- und Schwefelumsatzes. 2. N- und S- Stoffwechsel gesunder Säuglinge bei künstlicher Ernährung. Untersuchungen über die Ursache der Differenz in der Ausnützung des Frauen und Kuhmilcheiweisses. *Monatschr. Kinderheilk.*, 81: 214, 1940. Cited, *Nutr. Abst. Rev.*, 10: 355, 1940.

354. Van Liere, Edward J., Clark K. Sleeth, and David Northup. The relation of the size of the meal to the emptying time of the human stomach. *Am. J. Physiol.*, 119: 480, 1937.

355. Van Liere, Edward J., and Clark K. Sleeth. Studies in gastric motility; The relation of the size of the meal to the gastric emptying time in the dog, using a meal rich in fat and protein. *Am. J. Dig. Dis.*, 5: 18, 1938.

356. Van Slyke, Donald D., and Jordi Folch. Manometric carbon determination. *J. Biol. Chem.*, 136: 509, 1940.

357. Verzár, F., and E. J. McDougall. Absorption from the Intestine. Longmans, Green and Co., London, 1936.

358. Vickery, Hubert Bradford, and Abraham White. Proportion of cystine yielded by hemoglobins of the horse, dog and sheep. *Proc. Soc. Exper. Biol. Med.*, 31: 6, 1933.

359. Vickery, H. B. Evidence from organic chemistry regarding the composition of protein molecules. *Ann. N. Y. Acad. Science*, 41: 87, 1941.

360. Vigneaud, Vincent du, Joseph P. Chandler, Mildred Cohn, and George Bosworth Brown. The transfer of the methyl group from methionine to choline and creatine. *J. Biol. Chem.*, 134: 787, 1940.

361. Vigneaud, Vincent du. Interrelationships between choline and other methylated compounds. *In*, Biological Symposia, Vol. V, Comparative Biochemistry. Intermediate Metabolism of Fats. Carbohydrate Metabolism. Biochemistry of Choline. *Edited by*, Howard B. Lewis. The Jaques Cattell Press, Lancaster, Pa., 1941.

362. Vigneaud, Vincent du, Mildred Cohn, Joseph P. Chandler, Jay R. Schenck, and Sofia Simmonds. The utilization of the methyl group of methionine in the biological synthesis of choline and creatine. *J. Biol. Chem.*, 140: 625, 1941.

363. Voris, Leroy, Gordon Ellis, and L. A. Maynard. The determination of neutral fat glycerol in blood with periodate application to the determination of arteriovenous differences in neutral fat. *J. Biol. Chem.*, 133: 491, 1940.

364. Wang, Chi Che, Mildred Kaucher, and Margaret Frank. Metabolism of undernourished children. IV. Calcium metabolism. *Am. J. Dis. Child.*, 35: 856, 1928.

365. Wang, Chi Che, Jean E. Hawks, and Bertha B. Hays. Metabolism of undernourished children. V. Protein metabolism. *Am. J. Dis. Child.*, 35: 968, 1928.

366. Wang, Chi Che, and Mildred Kaucher. Metabolism of undernourished children. VIII. The effect of high and low protein diets on the excretion of creatine, creatinine and ammonia. *Am. J. Dis. Child.*, 38: 468, 1929.

367. Wang, Chi Che, Ruth Kern, and Mildred Kaucher. Metabolism of undernourished children. IX. A study of the basal metabolism, caloric balance and protein metabolism during a period of gain in weight. *Am. J. Dis. Child.*, 38: 476, 1929.

368. Wang, Chi Che. Value of quantitative methods in biochemistry in the study of growth and development. *Proc. Sec. Biennial Meeting, Soc. Res. Child Develop., Nat'l. Res. Council*, October 30, 1936.

369. Washburn, Alfred H. Blood cells in healthy young infants. I. The leukocytic picture during the first three months with special reference to hourly and daily variations. *Am. J. Dis. Child.*, 47: 993, 1934.

370. Washburn, Alfred H. Blood cells in healthy young infants. II. A comparison of routine and special technics in the differentiation of leukocytes. *Am. J. Dis. Child.*, 50: 395, 1935.

371. Washburn, Alfred H. Blood cells in healthy young infants. III. A study of 608 differential leukocyte counts, with a final report on 908 total leukocyte counts. *Am. J. Dis. Child.*, 50: 413, 1935.

372. Washburn, Alfred H. Blood cells in healthy young infants. IV. Postnatal readjustments of the red blood cells in individual babies. *Am. J. Dis. Child.*, 62: 530, 1941.

372*a*. Weech, A. A. A micro-photoelectric photometer. *Proc. Soc. Exper. Biol. Med.*, 45: 858, 1940.

373. West, Edward S., John R. Montague and Frederick R. Judy. Digestion and absorption in a man with three feet of small intestine. *Am. J. Dig. Dis.*, 5: 690, 1938.

374. Wetzel, Norman C. On the motion of growth. XV. Prolegomena to the clinical study of human growth and metabolism. *J. Pediatrics*, 3: 252, 1933.

375. Wetzel, Norman C. On the motion of growth. XVI. Clinical aspects of human growth and metabolism with special reference to infancy and preschool life. *J. Pediatrics*, 4: 465, 1934.

376. Wetzel, Norman C. On the motion of growth. XVII. Theoretical foundations. *Growth*, 1: 6, 1937.

377. Whipple, Dorothy V., and Charles F. Church. The composition of growth induced by vitamin B (B_1). *J. Biol. Chem.*, 114: cvii, 1936.

378. Whipple, Dorothy V., and Charles F. Church. The effect of Vitamin B_1 on the respiratory quotient. *J. Biol. Chem.*, 119: ciii, 1937.

379. Widdowson, Elsie May, and Robert Alexander McCance. The absorption and excretion of iron before, during and after a period of very high intake. *Biochem. J.*, 31: 2029, 1937.

380. Willard, Hobart H., and Lucien H. Greathouse. The colorimetric determination of manganese by oxidation with periodate. *J. Am. Chem. Soc.*, 39: 2366, 1917.

381. Willard, Hobart H., and N. Howell Furman. Elementary quantitative analysis. Theory and Practice. Second edition. D. Van Nostrand Co., Inc., New York, 1935.

382. Williams, Harold H., and William E. Anderson. The relation of lipids to physiological activity. *Oil and Soap*, 14: 122, 1937.

383. Williams, Harold H., Betty Nims Erickson, Samuel Bernstein, Frances Cope Hummel and Icie G. Macy. The lipid and mineral distribution of the serum and erythrocytes in pernicious anemia before and after therapy. *J. Biol. Chem.*, 118: 599, 1937.

384. Williams, Harold H., Betty Nims Erickson, Ira Avrin, Samuel S.

Bernstein and Icie G. Macy. Determination of cephalin in phospholipids by the estimation of choline. *J. Biol. Chem.*, 123: 111, 1938.

385. Williams, Harold H., Betty Nims Erickson, Samuel S. Bernstein and Icie G. Macy. Phospholipid partition (lecithin, cephalin and sphingomyelin) of blood in pernicious anemia and lipemia. *Proc. Soc. Exper. Biol. Med.*, 45: 151, 1940.

386. Williams, Harold H., Betty Nims Erickson, Eliot F. Beach, and Icie G. Macy. Biochemical studies of the blood of dogs with N-propyl disulfide anemia. *J. Lab. Clin. Med.*, 26: 996, 1940.

386*a*. Williams, Harold H., Betty Nims Erickson, and Icie G. Macy. Chemical structure of the red blood cell. *Quart. Rev. Biol.*, 16: 80, 1941.

387. Williams, Ray D., and W. H. Olmsted. A biochemical method for determining indigestible residue (crude fiber) in feces: Lignin, cellulose, and non-water-soluble hemicelluloses. *J. Biol. Chem.*, 108: 653, 1935.

388. Williams, Ray D., and William H. Olmsted. The effect of cellulose, hemicellulose and lignin on the weight of the stool: A contribution to the study of laxation in man. *J. Nutrition*, 11: 433, 1936.

389. Williams, Ray D., and W. H. Olmsted. The manner in which food controls the bulk of the feces. *Ann. Internal Med.*, 10: 717, 1936.

390. Williams, Robert R., and Tom D. Spies. Vitamin B_1 (Thiamin) and Its Use in Medicine. The Macmillan Co., New York, 1938.

391. Wilson, Edwin B. Heights and weights of two hundred and seventy-five public school girls for consecutive ages of 7 to 16 years inclusive. *Proc. Nat. Acad. Science*, 21: 633, 1935.

392. Wolfe, John K., E. B. Hershberg and Louis F. Fieser. The polarographic determination of ketonic steroids. *J. Biol. Chem.*, 136: 653, 1940.

393. Word, Aubrey H., and Glen Wakeham. Variations in mineral food analyses. *University of Colorado Studies*, 25: 181, 1938.

394. Wright, C. B. Gastric secretion, gastro-intestinal motility and position of the stomach in a group of 250 children of the Lymanhurst School. *Arch. Internal Med.*, 33: 435, 1924.

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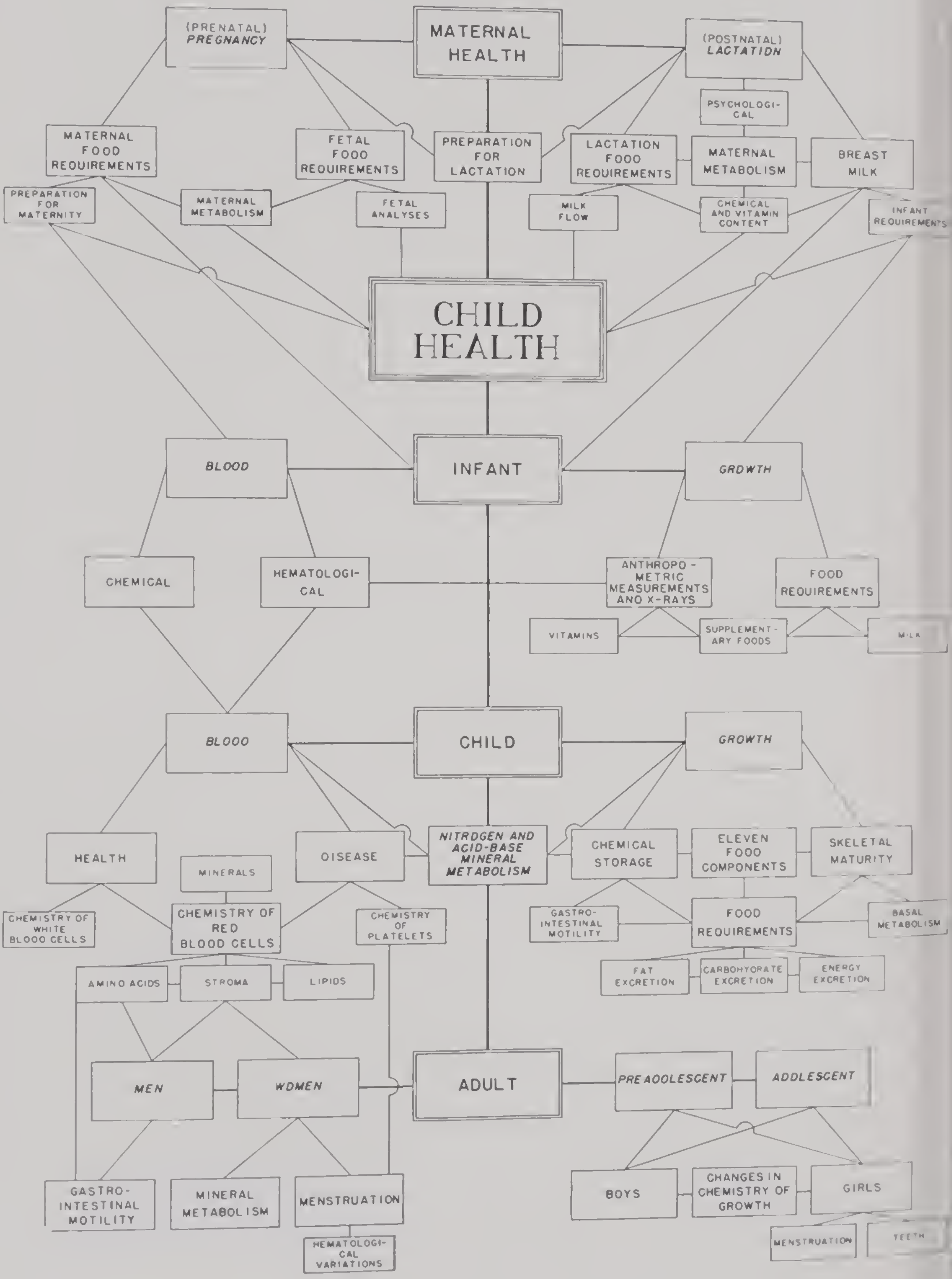

PAPERS PUBLISHED FROM

The Nutrition Research Laboratories of the Merrill-Palmer School and Children's Hospital of Michigan, and the Research Laboratory of the Children's Fund of Michigan

Composition and Secretion of Milk

Certain Biological Properties of Human Milk. B. Raymond Hoobler, Julia Outhouse, and Icie G. Macy. *Am. J. Dis. Child.*, 32: 456, 1926.

Comparative Vitamin Content of Human and Cow's Milk. Icie G. Macy. *J. Mich. State Med. Soc.*, 26: 669, 1927.

Observations Upon the Composition of Blood and Milk of Women During the Different Stages of Lactation. Icie G. Macy, Julia Outhouse, M. Louisa Long, Minerva Brown, Helen A. Hunscher, and B. Raymond Hoobler. *J. Biol. Chem.*, 74: *xxxi*, 1927.

Breast Milk—A Variable Food. Icie G. Macy and Julia Outhouse. *J. Am. Diet. Assoc.*, 4: 9, 1928.

The Variability in Vitamin Content of Human Milks. Icie G. Macy, Julia Outhouse, and Helen Hunscher. *J. Home Econ.*, 20: 897, 1928.

The Vitamin Content of Milk Used in Infant Feeding. Icie G. Macy and Julia Outhouse. *Am. J. Dis. Child.*, 37: 379. 1929.

Human Milk Flow. Icie G. Macy, Helen A. Hunscher, Eva Donelson, and Betty Nims. *Am. J. Dis. Child.*, 39: 1186, 1930.

Human Milk Studies:

I. Technique Employed in Vitamin Studies. Icie G. Macy, Julia Outhouse, M. Louisa Long, and Alice Graham. *J. Biol. Chem.*, 73: 153, 1927.

II. The Quantitative Estimation of Vitamin A. Icie G. Macy, Julia Outhouse, Alice Graham, and M. Louisa Long. *J. Biol. Chem.*, 73: 175, 1927. [*J. Biol. Chem.*, 67: *li*, 1926.]

III. The Quantitative Estimation of Vitamin B. Icie G. Macy, Julia Outhouse, Alice Graham, and M. Louisa Long. *J. Biol. Chem.*, 73: 189, 1927. [*J. Biol. Chem.*, 67: *li*, 1926.]

IV. A Note on the Vitamin A and B Content of Cow's Milk. Julia Outhouse, Icie G. Macy, Viola Brekke, and Alice Graham. *J. Biol. Chem.*, 73: 203, 1927.

V. A Quantitative Comparison of the Antiricketic Factor in Human Milk and Cow's Milk. Julia Outhouse, Icie G. Macy, and Viola Brekke. *J. Biol. Chem.*, 78: 129, 1928.

VI. Vitamin Potency as Influenced by Supplementing the Maternal Diet with Yeast. Sylvia Schimmel McCosh, Icie G. Macy, and Helen A. Hunscher. *J. Biol. Chem.*, 90: 1, 1931.

VII. Chemical Analysis of Milk Representative of the Entire First and Last Halves of the Nursing Period. Icie G. Macy, Betty Nims, Minerva Brown, and Helen A. Hunscher. *Am. J. Dis. Child.*, 42: 569, 1931.

VIII. A Comparison of the Composition of the Milk from the Two Breasts. Minerva Brown, Icie G. Macy, Betty Nims, and Helen A. Hunscher. *Am. J. Dis. Child.*, 43: 40, 1932.

IX. Variations in the Composition of Milk at Four Hour Intervals During the Day and Night. Betty Nims, Icie G. Macy, Minerva Brown, and Helen A. Hunscher. *Am. J. Dis. Child.*, 43: 828, 1932.

- X. Daily and Monthly Variations in Milk Components as Observed in Two Successive Lactation Periods. Betty Nims, Icie G. Macy, Helen A. Hunscher, and Minerva Brown. *Am. J. Dis. Child.* 43: 1062, 1932.
- XI. Vitamin G (B_2) Content of Mixed Milk. Eva Donelson and Icie G. Macy. *Am. J. Physiol.*, 100: 420, 1932.
- XII. The Vitamin B and Vitamin G Content Before and During Maternal Consumption of Yeast. Eva Donelson and Icie G. Macy, *J. Nutrition*, 7: 231, 1934.
- XIII. Vitamin Potency as Influenced by Supplementing the Maternal Diet with Vitamin A. Sylvia Schimmel McCosh, Icie G. Macy, Helen A. Hunscher, Betty Nims Erickson, and Eva Donelson. *J. Nutrition*, 7: 331, 1934.
- XIV. A Critique of the Determinations of Nitrogenous Constituents. Betty Nims Erickson, Neva Stoner, and Icie G. Macy. *J. Biol. Chem.*, 103: 235, 1933.
- XV. The Non-Protein Nitrogen Constituents. Betty Nims Erickson, Margaret Gulick, Helen A. Hunscher, and Icie G. Macy. *J. Biol. Chem.*, 106: 145, 1934.
- XVI. Vitamin D Potency as Influenced by Supplementing the Diet of the Mother During Pregnancy and Lactation with Cow's Milk Fortified with a Concentrate of Cod Liver Oil (A Test on Rachitic Infants and Rats). Donald J. Barnes, Frances Cope, Helen A. Hunscher, and Icie G. Macy. *J. Nutrition*, 8: 647, 1934.

The Nutritive Aspects of Human Milk. Icie G. Macy. *Yale J. Biol. Med.*, 4: 451, 1932.

Metabolism of Women

Metabolism of Women During the Reproductive Cycle:

- I. Calcium and Phosphorus Utilization in Pregnancy. Icie G. Macy, Helen A. Hunscher, Betty Nims, and Sylvia Schimmel McCosh. *J. Biol. Chem.*, 86: 17, 1930.
- II. Calcium and Phosphorus Utilization in Two Successive Lactation Periods. Helen A. Hunscher. *J. Biol. Chem.*, 86: 37, 1930. [*J. Biol. Chem.*, 78: *xxvi*, 1928.]
- III. Calcium, Phosphorus and Nitrogen Utilization in Lactation Before and After Supplementing the Usual Home Diets With Cod Liver Oil and Yeast. Icie G. Macy, Helen A. Hunscher, Sylvia Schimmel McCosh, and Betty Nims. *J. Biol. Chem.*, 86: 59, 1930.
- IV. Calcium and Phosphorus Utilization in Late Lactation and During Subsequent Reproductive Rest. Eva Donelson, Betty Nims, Helen A. Hunscher, and Icie G. Macy. *J. Biol. Chem.*, 91: 675, 1931.
- V. Nitrogen Utilization. Helen A. Hunscher, Eva Donelson, Betty Nims, Fanny Kenyon, and Icie G. Macy. *J. Biol. Chem.*, 99: 507, 1933.
- VI. A Case Study of the Continuous Nitrogen Utilization of a Multipara During Pregnancy, Parturition, Puerperium and Lactation. Helen A. Hunscher, Frances Cope Hummel, Betty Nims Erickson, and Icie G. Macy. *J. Nutrition*, 10: 579, 1935. [*J. Nutrition*, 9: Suppl., 13, 1935.]
- VII. Utilization of Inorganic Elements (A Continuous Case Study of a Multipara). Frances Cope Hummel, Helen R. Sternberger, Helen A. Hunscher, and Icie G. Macy. *J. Nutrition*, 11: 235, 1936.

Some Physiological Variations in Peripheral Blood Cell Counts. Fanny Kenyon and Icie G. Macy. *Am. J. Physiol.*, 93: 665, 1930.

Psychophysiological Studies:

- I. The Technique of Securing Quantitative and Coördinated Psychological and Physiological Data on Lactating Women in Their Usual Home Environment. Helen A. Hunscher, E. Lee Vincent, and Icie G. Macy. *Child Develop.*, 1: 15, 1930.

Simple Methods for Metabolic Balance Studies and Their Interpreta-

tion. Eva Donelson, Betty Nims, Helen A. Hunscher, Carroll F. Shukers, and Icie G. Macy. *J. Home Econ.*, 23: 267, 1931.

Nitrogen, Calcium and Phosphorus Balances in Late Gestation Under a Specified Dietary Régime. Icie G. Macy, Eva Donelson, M. Louisa Long, Alice Graham, Mary E. Sweeny, and Margaret Shaw. *J. Am. Diet. Assoc.*, 6: 314, 1931.

Food Intake in Pregnancy, Lactation, and Reproductive Rest in the Human Mother. Carroll F. Shukers, Icie G. Macy, Eva Donelson, Betty Nims, and Helen A. Hunscher. *J. Nutrition*, 4: 399, 1931.

Sources of Nutrients Chosen by Women During Pregnancy, Lactation, and Reproductive Rest. Carroll F. Shukers, Icie G. Macy, Eva Donelson, Betty Nims, and Helen A. Hunscher. *J. Am. Diet. Assoc.*, 7: 235, 1931.

A Quantitative Study of the Dietary of the Human Mother with Respect to the Nutrients Secreted into Breast Milk. Carroll F. Shukers, Icie G. Macy, Betty Nims, Eva Donelson and Helen A. Hunscher. J. *Nutrition*, 5: 127, 1932.

Results of the Ingestion of Cod Liver Oil and Yeast on Calcium and Phosphorus Metabolism of Women. Helen A. Hunscher, Eva Donelson, Betty Nims Erickson, and Icie G. Macy. *J. Nutrition*, 8: 341, 1934.

An Evaluation of Maternal Nitrogen and Mineral Needs During Embryonic and Infant Development. Icie G. Macy and Helen A. Hunscher. *Am. J. Obstet. Gyn.*, 27: 878, 1934.

A Consideration of the Nutritive State in the Metabolism of Women During Pregnancy. Frances Cope Hummel, Helen A. Hunscher, Mary F. Bates, Priscilla Bonner, and Icie G. Macy. *J. Nutrition*, 13: 263, 1937.

The Basal Metabolism in Pregnancy. Joseph A. Johnston, Helen A. Hunscher, Frances Cope Hummel, Mary F. Bates, Priscilla Bonner, and Icie G. Macy. *J. Nutrition*, 15: 513, 1938.

Hourly Physiologic Variations in Peripheral Hemoglobin, Red and White Cell Counts of Women: Effect of Pregnancy Upon Blood Counts. Fanny Kenyon and Icie G. Macy. *Human Biol.*, 10: 511, 1938.

Infant Growth

The Chemical Composition of the Human Fetus. Maurice H. Givens and Icie G. Macy. *J. Biol. Chem.*, 102: 7, 1933. [*J. Biol. Chem.*, 50: *xxxiv*, 1922]

A Controlled Study of the Nutritional Value of Prepared Foods in Infant Feeding. B. M. Hamil. *Pub. Health Nursing*, 28: 325, 1936.

The Interpretation of X-ray Films in the Diagnosis of Rickets. Thomas B. Cooley and Lawrence Reynolds. *J. Pediatrics*, 10: 743, 1937.

Stabilizing Effect of Increased Vitamin B (B_1) Intake on Growth and Nutrition of Infants. Marsh W. Poole, Brenton M. Hamil, Thomas B. Cooley, and Icie G. Macy. *Am. J. Dis. Child.*, 54: 726, 1937.

Relation of Increased Vitamin B (B_1) Intake to Mental and Physical Growth of Infants. Martha G. Colby, Icie G. Macy, Marsh W. Poole, Brenton M. Hamil, and Thomas B. Cooley. *Am. J. Dis. Child.*, 54: 750, 1937.

Relationship Between Vitamin D Intake and Linear Growth in Infants. Francis Slyker, B. M. Hamil, M. W. Poole, T. B. Cooley, and Icie G. Macy. *Proc. Soc. Exper. Biol. Med.*, 37: 499, 1937.

The Measurement of Variations in Asymmetrical Data With Application to Hemoglobin Statistics of Infants. T. E. Raiford. *Human Biol.*, 10: 136, 1938.

A Consideration of Quantitative Relations Between Erythrocytes, Leucocytes and Hemoglobin of the Blood. B. M. Hamil, M. W. Poole, Betty Munday, Marion L. Shepherd, Louise Emerson, Icie G. Macy, and T. E. Raiford. *J. Lab. Clin. Med.*, 23: 488, 1938.

Hemoglobin Differences in Healthy White and Negro Infants. Betty Munday, Marion L. Shepherd, Louise Emerson, Brenton M. Hamil, Marsh W. Poole, Icie G. Macy, and T. E. Raiford. *Am. J. Dis. Child.*, 55: 776, 1938.

Addition of Vegetable Soup and Strained Vegetables to Diet of Artifically Fed Infants. M. W. Poole, B. M. Hamil, T. B. Cooley, and I. G. Macy. *Am. J. Dis. Child.*, 55: 1158, 1938.

The Relationship Between Height and Weight of Male and Female Infants Between the Ages of Two Weeks and One Year. T. E. Raiford. *Human Biol.*, 10: 409, 1938.

Minimal Vitamin C Requirements of Artifically Fed Infants. Brenton M. Hamil, Lawrence Reynolds, Marsh W. Poole, and Icie G. Macy. *Am. J. Dis. Child.*, 56: 561, 1938.

Relation of Craniotabes to Diet and Growth. Marsh Poole. *J. Pediatrics*, 13: 936, 1938.

Metabolism of Children

Calcium and Phosphorus Storage in Growing Children. Helen A. Hunscher, Frances Cope, Alice Noll, and Icie G. Macy. *J. Biol. Chem.*, 100: *lv*, 1933.

Daily Fluctuations in Urinary *pH*. Fanny Kenyon, Charles A. Wilson, and Icie G. Macy. *Arch. Pediatrics*, 51: 490, 1934.

Mineral Metabolism. A Case of Osteopsathyrosis and One of Ununited Fracture. Thomas B. Cooley, Grover C. Penberthy, Lillian Armstrong, Helen A. Hunscher, Frances Cope, and Icie G. Macy. *Am. J. Dis. Child.*, 50: 431, 1935. [*J. Biol. Chem.*, 97: *lxiv*, 1932.]

Variability of Metabolic Response of Different Children to a Given Intake of Calcium. Helen A. Hunscher, Frances Cope Hummel, and Icie G. Macy. *Proc. Soc. Exper. Biol. Med.*, 35: 189, 1936.

Is the Metabolic Nitrogen Balance an Accurate Measure for Adequate Protein Consumption in Children? Icie G. Macy, Helen A. Hunscher, Frances Cope Hummel, Mary F. Bates, and Marsh W. Poole. *J. Nutrition*, 11: Suppl. 6, 1936.

Is Skeletal Maturity Related to Calcium Storage in Children? Helen A. Hunscher, Frances Cope Hummel, Icie G. Macy, T. Wingate Todd, and C. C. Francis. *J. Biol. Chem.*, 119: *lii*, 1937.

The Influence of a Daily Serving of Spinach or Its Equivalent in Oxalic Acid Upon the Mineral Utilization of Children. P. Bonner, F. C. Hummel, M. F. Bates, J. Horton, H. A. Hunscher, and I. G. Macy. *J. Pediatrics*, 12: 188, 1938.

Determination of Iron in Biological Materials. The Use of *o*-Phenanthroline. Frances Cope Hummel and H. H. Willard. *Ind. Eng. Chem.*, Anal. Ed., 10: 13, 1938.

Biological Time Patterns in Gastrointestinal Motility of Children. Lawrence Reynolds. *J. Pediatrics*, 13: 935, 1938.

Longitudinal and Cross-Sectional Data on Storage of Nitrogen and Calcium in Actively Growing Children. Icie G. Macy. *Am. J. Dis. Child.*, 56: 937, 1938.

Relation of Growth and Skeletal Maturity to the Mineral Balances of Children. Icie G. Macy. *J. Pediatrics*, 13: 935, 1938.

The Relative Effect of Different Type Milks Upon the Nitrogen and Acid-Base Mineral Retentions of Average Well Children. Marion L. Shepherd and Icie G. Macy. *Med. Woman's J.*, 46: 17, 1939.

The Effect of Carmine Upon the Gastrointestinal Motility of Children. Icie G. Macy, Lawrence Reynolds, and Helen J. Souders. *Am. J. Physiol.*, 126: 75, 1939.

Metabolic Observations on a Child with Essential Hyperlipemia. Samuel S. Bernstein, Harold H. Williams, Frances C. Hummel, Marion L. Shepherd, and Betty Nims Erickson. *J. Pediatrics*, 14: 570, 1939. [*J. Pediatrics*, 13: 936, 1938.]

Effect of Irradiated Milks on Storage of Nitrogen and Acid-Base Minerals in Children. Frances Cope Hummel, Helen A. Hunscher, and Icie G. Macy. *Am. J. Dis. Child.*, 58: 753, 1939.

Influence of Fluid and of Evaporated Milk on Mineral and Nitrogen Metabolism of Growing Children. Helen J. Souders, Helen A. Hunscher, Frances C. Hummel, and Icie G. Macy. *Am. J. Dis. Child.*, 58: 529, 1939.

The Influence of Different Levels of Milk Intake on the Acid-Base Mineral Balances of Children. Helen A. Hunscher, Frances C. Hummel, and Icie G. Macy. *J. Nutrition*, 17: 461, 1939.

The Gastrointestinal Response of Children to Test Meals of Barium and Pasteurized, Evaporated, and Base-Exchanged Milks. Lawrence Reynolds, Icie G. Macy, and Helen J. Souders, *J. Pediatrics*, 15: 1, 1939.

Normal Variation in the Gastrointestinal Response of Healthy Children. Icie G. Macy, Lawrence Reynolds, Helen J. Souders, and Mary Bates Olson. *Am. J. Roentgen. Rad. Therapy*, 43: 394, 1940.

The Gastrointestinal Response of Average Healthy Children to Test Meals of Barium in Milk, Cream, Meat and Carbohydrate Media. Lawrence Reynolds, Icie G. Macy, Helen Hunscher, and Mary Bates Olson. *Am. J. Roentgen. Rad. Therapy*, 43: 517, 1940.

Effect of Changes in Food Intakes Upon the Lignin, Cellulose and Hemicellulose Contents of Diets. Frances C. Hummel, Marion L. Shepherd, and Icie G. Macy. *J. Am. Diet. Assoc.*, 16: 199, 1940.

Effects of Simple Dietary Alterations Upon Retention of Positive and Negative Minerals by Children. Icie G. Macy, Frances Cope Hummel, Helen A. Hunscher, Marion L. Shepherd, and Helen J. Souders. *J. Nutrition*, 19: 461, 1940.

Influence of Raw Banana and Apple Upon Disappearance of Complex Carbohydrates From the Alimentary Tracts of Normal Children. Marion L. Shepherd, Frances Cope Hummel, and Icie G. Macy. *Am. J. Dig. Dis.*, 7: 248, 1940.

Synthesized, Processed and Natural Sources of Vitamin C in the Mineral Metabolism of Normal Children. Marion L. Shepherd, Icie G. Macy, Helen A. Hunscher, and Frances Cope Hummel. *J. Pediatrics*, 16: 704, 1940.

Effect of Varied Banana Intake on Nitrogen and Mineral Balances of Normal Children. Helen A. Hunscher, Frances Cope Hummel, and Icie G. Macy. *Am. J. Dis. Child.*, 60: 509, 1940.

Fruits in the Diets of Children. Harold H. Williams and Icie G. Macy. *J. Am. Diet. Assoc.*, 17: 136, 1941.

Indirect Polarographic Determination of Phosphorus in Biological Material. Adolph Stern. *Ind. Eng. Chem.*, Anal. Ed., 14: 74, 1942.

Analysis and Composition of Blood

The Lipid and Mineral Distribution in the Serum and Erythrocytes of Normal Children. Betty Nims Erickson, Harold H. Williams, Frances Cope Hummel, and Icie G. Macy. *J. Biol. Chem.*, 118: 15, 1937.

The Lipid and Mineral Distribution of the Serum and Erythrocytes in the Hemolytic and Hypochromic Anemias of Childhood. Betty Nims Erickson, Harold H. Williams, Frances Cope Hummel, Pearl Lee, and Icie G. Macy. *J. Biol. Chem.*, 118: 569, 1937. [*J. Biol. Chem.*, 109: *xxx*, 1935; *Am. J. Dis. Child.*, 50: 812, 1935.]

The Lipid and Mineral Distribution of the Serum and Erythrocytes in Pernicious Anemia. Harold H. Williams, Betty Nims Erickson, Samuel Bernstein, Frances Cope Hummel, and Icie G. Macy. *J. Biol. Chem.*, 118: 599, 1937.

A Method for the Preparation of Posthemolytic Residue or Stroma of Erythrocytes. Samuel S. Bernstein, Robert L. Jones, Betty Nims Erickson, Harold H. Williams, Ira Avrin, and Icie G. Macy. *J. Biol. Chem.*, 122: 507, 1938. [*J. Biol. Chem.*, 114: *xxxii*, 1936.]

Determination of Cephalin in Phospholipids by the Estimation of Choline. Harold H. Williams, Betty Nims Erickson, Ira Avrin, Samuel S. Bernstein, and Icie G. Macy. *J. Biol. Chem.*, 123: 111, 1938.

The Lipid Distribution of Posthemolytic Residue or Stroma of Erythrocytes. Betty Nims Erickson, Harold H. Williams, Samuel S. Bernstein, Ira Avrin, Robert L. Jones, and Icie G. Macy. *J. Biol. Chem.*, 122: 515, 1938. [*Am. J. Dis. Child.*, 54: 1192, 1937; *J. Biol. Chem.*, 114: *xxxii*, 1936.]

Ten Years of Treatment and Progress in a Case of Chronic Myeloid Leukemia. Marsh W. Poole, Betty N. Erickson, Harold H. Williams, H. J. Burkholder, and T. Leucutia. *J. Mich. State Med. Soc.*, 37: 993, 1938.

TOTAL AND DIFFERENTIAL COUNTS, RATES OF DISINTEGRATION, AND LIPID COMPOSITION OF PLATELETS. Pearl Lee and Betty Nims Erickson. *Am. J. Dis. Child.*, 56: 937, 1938.

PLATELET STUDIES IN MENSTRUATION AND HEMOPHILIA: TOTAL AND DIFFERENTIAL COUNTS, DISINTEGRATION RATES AND LIPID DISTRIBUTIONS. Pearl Lee and Betty Nims Erickson. *Proc. Soc. Exper. Biol. Med.*, 39: 264, 1938.

THE LIPID DISTRIBUTION OF HUMAN PLATELETS IN HEALTH AND DISEASE. Betty N. Erickson, Harold H. Williams, Ira Avrin, and Pearl Lee. *J. Clin. Invest.*, 18: 81, 1939. [*J. Biol. Chem.*, 123: *xxxiv*, 1938.]

THE AMINO ACID COMPOSITION OF ERYTHROCYTE POSTHEMOLYTIC RESIDUE OF FIVE MAMMALIAN SPECIES. Eliot F. Beach, Betty Nims Erickson, Samuel S. Bernstein, Harold H. Williams, and Icie G. Macy. *J. Biol. Chem.*, 128: 339, 1939. [*J. Biol. Chem.*, 123: *vi*, 1938.]

PLATELET STUDIES IN NORMAL MEN AND WOMEN (MENSTRUATING AND NONMENSTRUATING) AND SUBJECTS WITH BLEEDING DISORDERS. Pearl Lee, and Betty Nims Erickson. *J. Lab. Clin. Med.*, 8: 821, 1939.

MICROMETHODS FOR THE DETERMINATION OF SPHINGOMYELIN AND CHOLINE. Betty Nims Erickson, Ira Avrin, D. Maxwell Teague, and Harold H. Williams. *J. Biol. Chem.*, 135: 671, 1940.

PHOSPHOLIPID PARTITION (LECITHIN, CEPHALIN AND SPHINGOMYELIN) OF BLOOD IN PERNICIOUS ANEMIA AND LIPEMIA. Harold H. Williams, Betty Nims Erickson, Samuel S. Bernstein, and Icie G. Macy. *Proc. Soc. Exper. Biol. Med.*, 45: 151, 1940.

RELATION BETWEEN CEREBROSIDE AND NEUTRAL FAT CONTENTS OF BLOOD PLASMA, ERYTHROCYTES AND STROMA. Betty Nims Erickson, Helen J. Souders, Marion L. Shepherd, D. Maxwell Teague, and Harold H. Williams. *Proc. Soc. Exper. Biol. Med.*, 45: 153, 1940.

BIOCHEMICAL STUDIES OF THE BLOOD OF DOGS WITH N-PROPYL DISULFIDE ANEMIA. Harold H. Williams, Betty Nims Erickson, Eliot F. Beach, and Icie G. Macy. *J. Lab. Clin. Med.*, 26: 996, 1941.

CORRELATED HEMATOLOGIC, PHYSICAL, AND CHEMICAL STUDIES OF THE BLOOD AND STROMA IN POLYCYTHEMIA AND BEEF EMBRYO. Betty Nims Erickson, Olive Hoffman, Eliot F. Beach, Harold H. Williams, and Icie G. Macy. *J. Lab. Clin. Med.*, 26: 1492, 1941.

CHEMICAL STRUCTURE OF THE RED BLOOD CELL. Harold H. Williams, Betty Nims Erickson, and Icie G. Macy. *Quart. Rev. Biol.*, 16: 80, 1941.

Amino Acids

TOTAL SULFUR, CYSTINE, AND METHIONINE CONTENT OF BLOOD GLOBINS OF FIVE MAMMALIAN SPECIES. Eliot F. Beach, Samuel S. Bernstein, Frances C. Hummel, Harold H. Williams, and Icie G. Macy. *J. Biol. Chem.*, 130: 115, 1939.

POLAROGRAPHIC MICRODETERMINATION OF CYSTINE IN PROTEIN HYDROLYSATES. Adolph Stern, Eliot F. Beach, and Icie G. Macy. *J. Biol. Chem.*, 130: 733, 1939.

MICRODETERMINATION OF HOMOCYSTINE IN PURE SOLUTION WITH THE DROPPING MERCURY CATHODE. Adolph Stern and Eliot F. Beach. *Proc. Soc. Exper. Biol. Med.*, 43: 104, 1940.

DISTRIBUTION OF NITROGEN AND PROTEIN AMINO ACIDS IN HUMAN AND IN COW'S MILK. Eliot F. Beach, Samuel S. Bernstein, Olive D. Hoffman, D. Maxwell Teague, and Icie G. Macy. *J. Biol. Chem.*, 139: 57, 1941.

INTAKE OF AMINO ACIDS BY BREAST-MILK-FED INFANTS AND AMINO ACID COMPOSITION OF COW'S MILK AND HUMAN MILK. Eliot F. Beach, Samuel S. Bernstein, and Icie G. Macy. *J. Pediatrics*, 19: 190, 1941.

A GRAVIMETRIC METHOD FOR THE DETERMINATION OF METHIONINE. Eliot F. Beach and D. Maxwell Teague. *J. Biol. Chem.*, 142: 277, 1942.

Special Research Investigations

HORMONE INFLUENCE IN RICKETS. M. Boyd Kay, Icie G. Macy, and Carroll F. Shukers. *J. Mich. State Med. Soc.*, 29: 561, 1930.

STUDIES ON THE QUANTITATIVE ESTIMATION OF BILE PIGMENTS. E. A. Peterman and Thomas B. Cooley. *J. Lab. Clin. Med.*, 19: 723, 1934.

THE USE OF HYDROGEN PEROXIDE IN THE QUANTITATIVE ESTIMATION OF BILIRUBIN IN BILE. E. A. Peterman and Thomas B. Cooley. *J. Lab. Clin. Med.*, 19: 743, 1934.

A STUDY OF THE THERAPEUTIC VALUE OF YEAST. Florence A. Brown, Mary B. Campbell, Neva B. Stoner, and Icie G. Macy. *J. Am. Diet. Assoc.*, 10: 29, 1934.

DIFFRACTION STUDIES OF THE EFFECT OF SODIUM FLUORIDE AND PARATHORMONE UPON THE INCISORS AND TIBIAE OF RATS. Lawrence Reynolds, Kenneth E. Corrigan, Henrietta S. Hayden, Icie G. Macy, and Helen A. Hunscher. *Am. J. Roentgen. Rad. Therapy*, 39: 103, 1938.

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